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The research demonstrates that parathion is highly toxic to aquatic species, may accumulate in tissues, and may persist long enough to cause chronic damage. To fine ecologically "safe" levels allowable in aquatic systems, the long-term effects of parathion must be studied further. The objective of the present study was to find the greatest chronic levels of parathion that produce no harmful effects to brook trout, bluegills, fathead minnows, Daphnia magna, Gammarus fasciatus and Chrionomus tentans. For each species, the maximum acceptable toxicant concentration (MATC), defined by Mount and Stephan, was then related to the acute LC50. Because standardized bioassay techniques were used, the results of these tests may be correlated with those for other species and compounds.

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ACUTE AND CHRONIC PARATHION TOXICITY TO FISH AND INVERTEBRATES

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ABSTRACT

Acute and chronic toxicities of parathion (0, 0-diethyl 0, p-nitrophenyl phosphorothionate) were found for bluegill, Lepomis macrochirus (Rafinesque), brook trout, Salvelinus fontinales (Mitchell), fathead minnow, Pimephales promelas (Rafinesque), water flea, Daphnia magna Strauss, scud, Gammarus fasciatus Say, and midge, Chironomus tentans Fabricius. The 96-hour LC50 values were: bluegill - 0.51 mg/l, trout - 1.76 mg/l, minnow 1.6 to 0.5 mg/l, D. magna - 0.63 µg/l, G. fasciatus - 0.4 µg/l, and C. tentans - 31 µg/l. Deformities and depressed acetylcholinesterase were found in bluegills above 0.17 µg/l after 23 months exposure. Trout showed no ill effects at 7.2 µg/l but trout eggs had reduced hatchability at 32 µg/l. Minnows had reduced egg production and deformities at about 4 µg/l in 8-1/2 months. Bluegills had parathion residues of 5 to 25 times the chronic water concentrations, trout had several hundred times, and minnows had residue levels intermediate to the two other species. Chronically exposed fish appeared to be most sensitive to parathion at the juvenile or adult stages. The chronic no-ill-effect level for D. magna was 0.08 µg/l, for G. fasciatus less than 0.04 µg/l, and for C. tentans, less than 3.1 µg/l.

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SECTION I

CONCLUSIONS

1. Juvenile bluegills were found to have a 96-hour median lethal parathion concentration (LC50) of 0.51 mg/l and a maximum acceptable toxicant concentration (MATC) of 0.17 to 0.34 µg/l based on adult deformities.

2. Brook trout had a 96-hour LC50 of 1.76 mg/l. Chronic levels of 7.2 µg/l produced no harmful effects. However, levels of 32 µg/l reduced hatchability of embryos from untreated parents.

3. Fathead minnows had 96-hour LC50 values between 1.6 and 0.5 mg/l and a chronic MATC of about 4 µg/l based on egg production and adult deformities.

4. Daphnia magna had a 96-hour LC50 of 0.63 µg/l and MATC of about 0.08 µg/l.

5. Gammarus fasciatus had a 96-hour LC50 of about 0.4 µg/l and a MATC of much less than 0.04 µg/l, which caused mortalities in one month.

6. Chironomus tentans had a 96-hour LC50 of 31 µg/l based on parathion concentration in water, and a MATC of less than 3.1 µg/l, a lethal level in two weeks.

7. Bluegill tissue concentrated chronic parathion residues at 5 to 25 times the water concentration, brook trout several hundred times, and minnows at rates intermediate to the other two species. No parathion above background levels was detected in D. magna.

8. Chronically exposed fish appeared to be most sensitive to parathion during juvenile or adult stages. The egg and fry stages were not particularly sensitive.

SECTION II

RECOMMENDATIONS

1. Parathion applications to water bodies should be limited to levels acutely tolerated by crustaceans such as Daphnia.

2. Trout and other species collected from parathion-treated sites should be examined further for potentially harmful residue levels.

3. The selectivity, toxicity, and persistence of alternative insecticides should be compared to parathion for possible substitution.

4. Food chain dynamics of parathion distribution in aquatic communities should be examined.

5. Metabolic pathway studies of parathion degradation in fish should relate effects of temperature, rates of reaction, and proportion of major metabolites.

6. Further histological characterization of parathion damaged tissues in fish would help to determine the mechanisms of such damage.

7. Further study is needed to insure bluegill spawning during chronic tests.

8. Five month chronic tests on Gammarus are not practical unless better survival rates are achieved in culture.

9. More efficient techniques are needed for performing the egg incubation and larval survival phases of chronic bioassays.

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SECTION III

INTRODUCTION

Among organophosphorus insecticides, parathion is one of the most persistent and toxic. About 15 million pounds of it are used annually for control of insect pests on fruit trees, cotton, tobacco, and other crops.[1] A small portion reaches aquatic environments by direct application (such as for mosquito control), land runoff, leaching, erosion, irrigation, aerial spraying, and spills during manufacturing, formulation, and application. Once in a water-soil system, its rate of degradation is variable, depending on pH, organic content, and microbial activity. Upon application to soil, parathion may persist for 3 months,[2] 5 years,[3] or in an extreme case, 16 years.[4] In distilled water of pH 7.4 the hydrolysis reaction has a half-life of about 108 days, decreasing with greater pH and temperature.[5] In contrast, natural river water degrades 50% of parathion within a week.[6] In natural lake sediments Graetz et al[7] found a half-life of 178 to 68 days.

Damage to aquatic fauna has been reported for parathion residues in water and sediments. After spraying an orchard, Nicholson[8] found reduced populations of aquatic insects in nearby ponds containing 1.9 ppm parathion in mud. Killifish and freshwater mussels exposed in a treated cranberry bog showed uptake of parathion residues.[9] Parathion spills have also caused kills of fish and amphibians.[10]

Like all organophosphates, parathion disrupts the central and peripheral nervous systems of animals when enzymatically converted to its oxygen analog, paraoxon. Paraoxon then combines covalently with acetylcholinesterase (AChE) to block the hydrolysis of acetycholine, the transmitter substance in the synapse of motor and parasympathetic nerves. A full account of this inhibition is given by O'Brien.[11] The AChE-phosphate complex is, at first, hydrolyzable. Animals may recover from acute doses of the inhibitor. However, the AChE-complex can gradually dealkylate, forming the phosphate anion which resists hydrolysis. Therefore, regeneration of AChE after long-term exposures of parathion is extremely slow.

Weiss demonstrated AChE activity in fish,[12] and its susceptability to paraoxon inhibition.[13] Bluegills exposed to 0.1 mg/l parathion for 8 hours recovered only 50% of normal activity in 30 days. Fathead minnows had a somewhat quicker response and recovery. Carter[14] found that channel catfish with 50% inhibition from methylparathion required 20 to

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30 days to regain 80% of normal activity. Studies of AChE inhibition *in vitro* have found fish[15, 16, 17] and lobsters[18] to be less sensitive than birds or mammals. Yet parathion is very toxic to both fish and crustaceans. The two principal metabolic products in rats[19] - paraoxon and diethyl hydrogen phosphorothionate have also been found in rainbow trout.[16] The proportion of the two metabolites formed, and their rates of formation vary with species. Metabolism can be slow enough to permit accumulation of the parathion in tissues, as reported for trout,[16] brown bullheads,[20] mosquitofish,[21] fathead minnows,[22] and oysters.[23]

Literature values for the acute toxicity of parathion to aquatic species have been summarized by Kempt, Abrams, and Overbeck.[24] Only a few studies have considered long-term effects. Mount and Boyle[20] found convulsions in bullheads exposed to 0.03 mg/l parathion for one month. Matsue[25] observed retarded growth of goldfish at 0.1 mg/l for one month. Reduced growth rates of oyster larvae in 0.05 mg/l were found by Davis and Hidu.[26] After 40 days at 0.01 mg/l, male guppies tested by Billard[27] showed reduced spermatogenesis. Jensen and Gaufin[28] reported a 30-day LC50 of 2.2 µg/l for *Pteronarcys californica* and 0.44 µg/l for *Acroneuria pacifica*.

Such work demonstrates that parathion is highly toxic to aquatic species, may accumulate in tissues, and may persist long enough to cause chronic damage. To find ecologically "safe" levels allowable in aquatic systems, the long-term effects of parathion must be studied further. The objective of the present study was to find the greatest chronic levels of parathion that produce no harmful effects to brook trout, bluegills, fathead minnows, *Daphnia magna*, *Gammarus fasciatus*, and *Chrionomus tentans*. For each species, the maximum acceptable toxicant concentration (MATC), defined by Mount and Stephan,[29] was then related to the acute LC50. Because standardized bioassay techniques were used, the results of these tests may be correlated with those for other species and compounds. The combined information will facilitate the prediction of chronic no-effect levels in freshwater.

SECTION IV

MATERIALS AND METHODS

WATER SUPPLY AND ENVIRONMENTAL CONTROL

Water Supply

Water for acute and chronic testing came directly from a 50 ft well located on the Tarrytown, N.Y. laboratory site. An analysis of the water composition is given in Table 1. Analytical methods for these results followed Standard Methods.[30] From the well, water was pumped to the laboratory through black iron and PVC pipe. At the laboratory the water was sterilized with ultraviolet light, and proportioned into lines of ambient (11-13°C) and heated water for distribution to testing stations. When heated, the hard water precipitated carbonate solids in the piping and steam heat exchanger. Therefore, part way through the program the heated water was softened by exchange of sodium for calcium. The hardness dropped about 20% in the bluegill chronic exposure, the only test underway at the time.

This system did not provide supplemental chilling. It was not installed because ambient water temperature the previous winter was about 9°C, the optimum for brook trout egg incubation. However, during the actual brook trout test, the water temperature was as high as 13°C in winter.

Temperature Control

Lines of both hot and cold water fed each test. Proportional mixing of the two flows was regulated by pneumatic temperature controller with sensing element in the mixing zone of the pipe. The resulting temperature variation was within ±1°C for instantaneous changes, and±2°C for daily trends. The actual temperature in at least one chamber per test was continuously monitored by thermistor probe read by a 20-channel electronic temperature monitor. Each channel had individual high and low set points which activated an alarm if exceeded. Once per hour all channels were recorded on punch paper tape and teletype.

Aeration

The well water coming directly from the piping was sometimes over or under-saturated with gases. For this reason water was aerated by air-stone as it entered the first proportional diluter cell. The adult fish chambers, particularly trout, had supplemental aeration to insure dissolved

Table 1 . ANALYSIS OF TEST WATER

(mg/l)

Component	Concentration	Component	Concentration
Alkalinity (as $CaCO_3$)	147	Lead	<0.0007
Aluminum	0.01	Magnesium	27
Ammonia (as N)	0.15	Manganese	0.005
Arsenic	<0.017	Mercury	< .0002
Barium	0.10	Nitrate (as N)	1.7
Boron	0.02	Nitrite (as N)	0.007
Cadmium	0.0005	Phenol	<0.0016
Calcium	76	Phosphorus	0.005
Chloride	130	Potassium	0.6
Chlorine	<0.001	Silicon	6
Chromium	<0.006	Sodium	3.9
Copper	0.03	Sulfate	39
Cyanide	<0.0001	Strontium	0.6
Fluoride	0.02	Tin	0.01
Hardness (as $CaCO_3$)	297	Titanium	0.01
Iron	<0.006	Zinc	< .001

Taken 3/10/72 and 10/19/72

oxygen levels greater than 60% saturation.

Toxicant Delivery

Reagent grade parathion (0, 0-diethyl 0-p-nitrophenyl phosphorothionate) of 99% purity (Pfaltz and Bauer) was used throughout. A stock solution of parathion in water or acetone was injected into a proportional diluter of the Mount and Brungs design.[31] Depending on the test, several injection devices were used: Sage 341 syringe pump, Mariotte bottle with constant flow, mechanical syringe pump,[31] or LKB Perplex peristaltic pump. The latter two devices were most reliable and precise over long periods of time. The peristaltic pump had the advantage of an unlimited stock solution reservoir, and was equal in precision to the mechanical syringe as long as the cycle time of the diluter remained constant. To prevent accidental injections during low-flow conditions, the electrical pumps were wired to stop if the diluter did not cycle.

All parts of the diluter in contact with test water were of glass, latex or Tygon tubing, and silicone glue. The diluter produced five concentrations of parathion, plus a control, each split into duplicate flows by a mixing cell. Individual delivery tubes went to each duplicate tank. Tanks were designated by the same number as the corresponding diluter cell:[31] "0" for the control, "1" for the highest toxicant concentration used, "2" for the second highest, up to "5" for the lowest. Duplicate tanks were designated "A" or "B".

The tank sizes and flow rates for each chronic test are summarized in Table 2. Bluegill adult tanks were made of No. 306 stainless steel. Trout, minnow and Gammarus adult tanks were commercial aquaria of glass with slate bottoms and outside stainless reinforcement. All other containers were glass with silicone glue (Dow Corning) construction.

Lighting

All tests were illuminated by Duro-Test Optima fluorescent lamps. These were set with a Tork timer to give the Evansville, Indiana, photo-period shown in Table 3. The timer wiring was modified to give 30 minutes of gradual change at dawn and dusk.[37] Daphnia and midges were held on a constant 16-hour light cycle. Average light intensities at the test sites were: bluegill, 75 ft-c; brook trout 70 ft-c; minnow 82 ft-c; and invertebrates 70 - 80 ft-c. The test photoperiods for bluegills and trout corresponded to the actual calendar. The minnow and invertebrate tests were begun on the dates shown in Table 3, but these did not correspond to the calendar.

Table 2. SUMMARY OF CHRONIC TEST CONDITIONS

Test animal	Adult tank volume, l	Minimum flow per tank, l/hr	Larval chamber volume, l	Temperature, °C
Bluegill	170	40	6[a]	25
Brook trout	85	30	6[a]	15-9
Fathead minnow	28	10	7[a]	28
Daphnia	3	0.8	-	18
Gammarus	15	0.5	-	18
Chironomus	3	0.8	-	25

a Two such chambers were used per adult tank.

Minnow chambers were not true duplicates. See page 9.

Table 3. CHRONIC TEST PHOTOPERIOD AND SCHEDULE

Period of light includes 30 min
of dawn and 30 min of dusk

Date	Light period, hr:min	Event
Jan. 1	10:30	
15	10:45	
Feb. 1	11:15	-Begin Gammarus
15	11:45	
Mar. 1	12:15	-Begin brook trout test
15	13:00	
Apr. 1	13:30	
15	14:15	Gammarus reproduction
May 1	14:45	
15	15:15	Fathead minnow spawning
June 1	15:30	
15	15:45	
July 1	15:45	Bluegill spawning
15	15:30	
Aug. 1	15:00	
15	14:30	
Sept. 1	14:00	
15	13:30	
Oct. 1	12:45	-Begin bluegill test
15	12:15	
Nov. 1	11:30	-Brook trout spawning
15	11:00	
Dec. 1	10:45	-Begin fathead test
15	10:30	

FISH BIOASSAYS

Bluegill

One stock of immature bluegills 5 to 8 cm long was collected from a pond in Duchess Co., N.Y., and acclimated in the laboratory for three months prior to the chronic testing. Other immature fish, taken from the same pond one year later, were used for the acute studies. Fish of both stocks had few internal parasites and a small number of gill parasites which were eradicated with formalin.

For the acute tests, an aqueous emulsion of 180 g parathion and 9 g Triton X-100 (Rohm and Haas) per liter of water was sonicated, and then injected into a proportional diluter by peristaltic pump. Each of the 85 liter test chambers received a flow of 30 l/hr. Acclimated test fish were randomly distributed at the start. Mortalities were counted and removed at regular intervals throughout the 96-hour tests. No aeration or feeding took place. Direct analysis of parathion in water was made at least once during each test.

The chronic exposure followed the "Recommended Bioassay Procedure for Bluegill Lepomis macrochirus (Rafinesque) Partial Chronic Tests" of the National Water Quality Laboratory, Duluth, Minn. (revised January 1972). The tanks had a flow of 40 l/hr and supplemental aeration. A stock solution of 2 to 4 g parathion, and 0.24 g Triton X-100 per liter acetone was injected by syringe pump. The nominal concentrations chosen for the test were originally 8.0, 4.0, 2.0, 1.0, and 0.5 μg/l.

On Dec. 22, 1971, 20 fish were randomly distributed to each tank. During the first two months of exposure, the fish at 8.0 μg/l showed tremors and convulsions. Therefore after 64 days, that concentration was removed while a 0.25 μg/l level with new fish was added. Fish were fed Oregon Moist Pellets and Silver Cup trout food ad libitum. Lengths and weights were recorded at the start, at thinning, and at termination of the adults.

Spawning did not occur the first summer, although the fish appeared mature. In the second summer season the males developed territorial behavior. Then each tank was thinned to 3 males and 7 females. All discarded fish were frozen at -18°C for later chemical analysis. A cement substrate, 27 cm square by 5 cm thick with a bowl-like depression, was placed at each end of each tank. To reduce aggressive male behavior, the tanks were divided into thirds by two black plastic curtains (53 cm wide by 40 cm long). These did not restrict mobility but reduced disturbance to the nests.

During spawning, the nests were checked for eggs daily. Spawned eggs were removed from the cement by gentle brushing, and extra eggs were siphoned from the tanks. Counting was done volumetrically, by counting the eggs in one milliliter, then determining the total volume of eggs. Two hundred eggs were incubated in an egg cup (4 oz. glass jar with 40 mesh nylon screen across the open bottom) suspended from a rocker arm in the larval chamber. The larval chamber received water from the corresponding parental tank. After three days the number of hatched larvae was counted, and 50 fry were retained in the cup. Feeding with large amounts of newly hatched Artemia salina (San Francisco Bay Brand) 4 times daily began on the 4th day after the spawn. At 7 days after spawn, larvae were released to a 18 x 38 x 13 cm wide larval chamber with a water depth of 13 cm. Mortalities were subsequently checked weekly. Thirty, 60 and 90 days after spawning, the surviving larvae were counted and measured photographically in an all-glass box set over a lightbox with millimeter transparent grid.[33]

All remaining adults were removed after 23 months exposure, examined, and frozen for residue analysis.

Brook Trout

Yearling brook trout, Salvelinus fontinales (Mitchell) from a hatchery in Sullivan Co., N.Y., approximately 60 g each, were acclimated at 12°C for three weeks prior to testing. Until the start of the chronic and acute tests, they showed no symptoms of disease and were not chemically treated. During the chronic test, fungus and furunculosis were treated with 20 ppm formalin, 4 ppm Furpyrinol, or 40 ppm malachite green.

The acute test method followed that for bluegills. An aqueous emulsion of 0.05 g Triton X-100 per gram of parathion was injected into the diluter by peristaltic pump. Water for parathion analysis was sampled at the start, and at 2, 4, and 5 days. Selected fish were frozen at -18°C for later residue analysis.

The chronic brook trout exposure followed the procedure given by the National Water Quality Laboratory.[34] On May 1, 1972, 14 trout were randomly distributed to each of 12 tanks. The diluter and flow were identical to those in the acute test. A stock solution of 8.0 g parathion and 0.24 g Triton X-100 per liter acetone was injected into the diluter by syringe pump. The maximum theoretical acetone concentration was 1.5 mg/l in the highest concentration used.

Trout were fed Oregon Moist Pellets (Moore-Clark Co.) according to the manufacturer's recommended ration. Weights and total length were taken at the start, at 117 days, at 178 days (discards only), and at the end, 282 days. At 178 days, the number in each tank was reduced to 7 males and 4 females. Secondary sexual characteristics made it possible to distinguish the sexes of most but not all of the fish. Discards were dissected and the tissues frozen for residue analyses.

On November 22, at 206 days, two polyethylene basins, 25 x 30 x 15 cm deep were placed in each tank as spawning substrates. These had nylon screening at each end to permit water circulation, and 5 cm of coarse gravel in the bottom. These substrates were difficult to handle and clean. Two weeks later the gravel was replaced by a horizontal layer of stainless steel screening about 5 cm off the bottom. Rocks were glued to the screen. These artificial substrates were far easier to handle and eggs easier to locate. Work by Benoit[35] has since showed that this type of substrate will jeopardize the fertilization of the eggs unless a baffle is added to the bottom to prevent eggs from rolling away from the spawning site.

Eggs were removed from the substrates each afternoon, and counted manually. Fifty eggs from each spawn were hatched in egg cups suspended in shaded larval chambers. The chambers received 7.6 l/hr directly from the mixing cells which supplied the parental tanks. Other eggs from each spawning were incubated for 12 days and examined for

viability. Under low magnification the embryonic development could be seen when the eggs were preserved in an aqueous solution of 5% formalin, 4% glacial acetic acid, and 6% glycerin (V/V). Viability was calculated as the percent of embryos with a neural keel. During incubation, the eggs were checked daily for mortalities and fungus. Insufficient larvae were hatched to carry out the larval exposure planned for this part of the test.

On Feb. 6, after 282 days, the remaining adult trout were removed. All but two from each tank were killed immediately and frozen at -4° to -18°C. The others were held in flow-through tanks of clean water for 2 to 7 days. Parathion residues were measured to show whether chronic parathion uptake was reversible.

To supplement the hatchability data from the chronic test, additional brook trout embryos from the Nashua National Fish Hatchery, Nashua, N.H. were exposed to parathion levels of 10, 32, and 100 µg/l with a control, at 10°C. Toxicant solutions of these concentrations were delivered at rates of 300 ml/hr to each of four larval chambers by constant drip Mariotte bottles. Each chamber incubated two egg cups containing 100 19-day-old embryos each. These were held until hatch. Every other day, the embryos were treated with a 4 mg/l solution of malachite green for one hour to prevent fungus.

Fathead Minnow

A stock of fathead minnows, Pimphales promelas (Rafinesque), originally from the Newtown Fish Toxicology Station, Cincinnati, Ohio, were cultured for two years in the laboratory and in an outside pond. All test fish were taken from this stock. Acute tests were performed as for bluegills. An aqueous emulsion of 29.0 g parathion and 1.45 g Triton X-100 per liter water was made by sonication and injected by peristaltic pump. The flow rate to each 10 liter test tank was about 15 l/hr in all acute tests.

The chronic exposure followed the procedure of the National Water Quality Laboratory.[34] A total of 35 minnow larvae, 5 to 14 days old, were distributed randomly to each tank on April 1 (Dec. 1 test date). For the first month they were fed powdered fish food, mixed with green algae, and Artemia nauplii. From then on, they were fed trout starter mash ad libitum.

Nominal parathion concentrations of 50, 25, 12, 6, and 3 µg/l were produced from a stock solution of 1.7 parathion and 0.1 g Triton X-100 per liter acetone.

At 30 and 60 days the minnow larvae were counted and photographically measured.[33] At 70 days each tank was thinned to 15 fish and three artifical substrates were added. These were made from 3-inch asbestos and cement drain tiles cut in half longitudinally.

Spawning began on Oct. 22 (June 22, test date) and continued through Dec. 19 (Aug. 19, test date) when the adults were terminated. During spawning, eggs were removed and counted daily. Fifty eggs from each spawning were incubated in an egg cup suspended in a larval chamber receiving water from the parent tank. Each larval chamber was 900 cm^3 by 15 cm deep divided into two sections. These two halves were not true duplicates, since they received the same water, but they did separate larvae spawned at different times. All egg cups were treated once with a solution of 4 mg/l malachite green to prevent fungal problems. Larvae were counted after hatching was completed, usually in 5 days. Of those hatched, 40 were released into the chamber for 30-day survival tests. Because a large excess of eggs was produced in the controls, some of these were also hatched at high parathion concentrations. At 30 days all larvae were counted and measured photographically.[33] The adult minnows were killed after 8-1/2 months of exposure, examined for deformities, measured, and frozen at -18°C for residue analysis.

INVERTEBRATE BIOASSAYS

Daphnia magna

A stock of Daphnia magna Strauss from the National Water Quality Laboratory, Duluth, Minn., was continuously cultured throughout the project. Groups of daphnids were held in 3 liter jars which had become coated with diatoms and other algae. No flow was required to maintain the cultures. A temperature of 18 to 21°C was suitable for reproduction. A food supply of 1.5 g Cerophyl, 2.0 g trout mash, and 1.0 g yeast in 500 ml well water was given periodically. When culture jars became too filled with detritus, about once a month, the daphnids were transferred to clean jars.

Before the acute or chronic test, several dozen large adults were selected and held in clean 250 ml beakers overnight. Within 24 hours enough first instar daphnids were usually produced to begin a test. These were counted and randomly distributed to the test containers. In an acute test, the number of surviving Daphnia were counted daily, and in a chronic, weekly. Young produced during the longer tests were counted and removed weekly. Size differences made it possible to distinguish the original animals from their offspring. Daphnia in all tests were fed 5 ml of the food mixture per tank daily.

Test jars had a U-shaped notch cut in the upper lip, covered with No. 405 Nytex screening to maintain a constant water level without losing test animals. Jars were held in an 18°C water bath for temperature control. Each jar received a separate stream of water (0.8 l/hr) from the toxicant diluter.

Gammarus fasciatus

Adult and immature Gammarus were collected in coves and tributaries of the Hudson River in an area above the salt wedge. The most successful collection methods were wire cage traps weighted with rocks, and dip nets. There are several very closely related Gammarus species in this area. Subsamples of the population used for testing were identified as G. fasciatus by Dr. E. L. Bousfield of the National Museum of Canada, Ottawa, Ontario, and by Dr. S. Ristich of Boyce Thompson Inst., Yonkers, N.Y. Because immature and female gammarids are extremely difficult to identify, we cannot exclude the possibility that some other species were included in the test population of G. fasciatus.

Tests were started with young gammarids collected from the field because laboratory cultures did not produce sufficient young. Gammarids were raised in the laboratory by confining two paired adults to a small, static container until the offspring were produced. The adults were immediately removed to avoid possible cannibalism of the young.

All tests were performed in 15-liter aquaria held at 18 to 20°C Each tank was supplied with several square centimeters of well-soaked maple leaves for cover and food. During chronic tests and culturing the Gammarus were fed small cubes of beef liver daily. This food source gave higher survival rates than dried fish food, aquatic macrophytes, or maple leaves alone.

The proportional diluter was supplied with a 200 mg/l stock solution of parathion in acetone. The maximum possible acetone concentration was about 7.5 mg/l in acute and chronic tests.

Originally a 5-month chronic exposure starting with newly hatched gammarids was planned for this project. The chronic test was started five times and each time encountered difficulties in survival or parathion level.

However, two sub-chronic exposures of juvenile gammarids for 35 and 43 days duration were completed, with estimates of the LC50 levels for these times.

Chironomus tentans

A stock of Chironomus tentans Fabricius provided by Dr. William Cooper of Michigan State University was cultured according to his methods. Several 2-liter aquaria with screen covers were filled halfway with water. A substrate of Cerophyl, trout food, and toilet tissue was blended together until lumpy and added in small amounts. It was important to aerate gently to avoide anaerobic conditions and yet leave the substrate undisturbed. For culturing, a photoperiod of 16 hours of light was used; a dawn and dusk period was not necessary. The static culture tanks were held at 21 to 25°C. Once a day, the emerged adults were transferred to a large screened cage which had shallow dishes of water covering the bottom. After mating, the females deposited their eggs in these pans where they were easily detected and removed. Egg cases produced in this way were used to begin tests or returned to fresh culture tanks.

Acute tests were performed with fourth instar larvae, the last stage of development before pupation. Sub-chronic tests were started with second instar larvae, about one week old. These were much easier to handle and count than first instars and did not suffer as many losses from handling. All tests were conducted in a 21°C water bath, in 3-liter battery jars similar to those used for Daphnia. Two sub-chronic tests, originally planned for one month each, were ended after two weeks because of mortality at all parathion levels. The lack of response of larvae to probing was interpreted as mortality.

CHEMICAL ANALYSIS

Samples for routine water quality measurements were taken by siphoning or dipping water from the center of each tank, away from the sides, top, or bottom. Dissolved oxygen samples were taken in BOD bottles and measured with a Yellow Springs Instruments polarographic oxygen meter. Hardness, alkalinity, and pH were determined by Standard Methods.[30]

Water for parathion analysis in fish tanks was sampled in the same way. For invertebrate tests, it was taken directly from the diluter and pooled, to avoid trapping test animals with the water. During long term tests, parathion analysis was made weekly for all tanks. Daily samples were pooled weekly in the case of the minnow test. Analysis of samples stored for one week in amber glass bottles were not significantly different from replicates analyzed immediately. Depending on parathion concentration expected, from 90 to 900 mls of water were extracted in volumetric flasks with 5.0 ml pesticide-grade hexane by 15 minutes of stirring. The hexane layer was allowed to separate and was removed by pipette. Duplicate aliquots were analyzed by gas chromatography. The gas chromatographic parameters for the Hewlett-Packard 5700 or 7620 series instruments used were:

Detector: Ni-63, Electron Capture, 265°C
Injection Port: 230°C
Column: 4 ft. x 1/4 in. 3% OV - 17 on 80 - 100 mesh Chromosorb W at 200°C
Carrier Gas: 10% methane in argon; flow: - 50 ml per min through column, 50 ml per min, detector purge
Injection Size: 10 microliters or suitable volume
Response Factors: Parathion - 45000 counts per nanogram
Paraoxon - 20000 counts per nanogram
System noise level (as parathion) based on hexane blanks: 0.001 to 0.02 ng of parathion on column

The same GC conditions were used for hexane extracts of water and fish blood, and fractions of tissue extracts after clean-up.

Fresh fish whole blood, drawn from the caudal vein, was extracted by shaking with 5 ml of hexane per ml blood and analyzed by GC without clean-up. This method produced some interferences, but its speed and simplicity recommended it over a long clean-up procedure. All other biological tissues were preserved at - 18°C until analysis. Two grams of the frozen tissue were blended with 60 g anhydrous sodium sulfate, and extracted overnight with 50 ml pesticide-grade hexane. The sodium sulfate was filtered, washed, and the hexane washes pooled (about 20 ml). The solvent was evaporated at 60 to 70°C just until

dryness. The residue was then redissolved and transferred to a silicic acid column (5 ml of 100 mesh Mallinkrodt chromatography grade) with 1 to 2 ml hexane. The column was washed with 20 ml hexane, and then eluted with 30% v/v benzene in hexane. A 25 ml benzene-hexane fraction was collected beginning at the 30% benzene break through (~4 ml) for low level parathion residues (in the µg/kg range). A 50 ml fraction was collected for higher parathion levels. The effluent was diluted or concentrated, as required, and analyzed by GC.

For fish tissues such as muscle, the reproducibility of analyses on replicate aliquots of the same fish was ±5 to 7%. Recovery of spiked samples averaged 90.8±7.6% based on seven tests. The range of recoveries was 83.1 to 103%; the coefficient of variation was 8.4%.

For acetylcholinesterase determinations, whole fish brains were dissected, stored up to 2 months in liquid nitrogen (-196°C), thawed, homogenized in phosphate buffer and assayed by the kinetic colorimetric method of Ellman et al.[38]

Statistical Methods

Whenever two or more partial kills occurred in an acute bioassay, the LC50 and 95% confidence limits were calculated by the graphical method of Litchfield and Wilcoxon.[36] Mortalities were plotted on a probit scale against the log of toxicant concentration. A straight line was fitted to the points by eye, and checked by the Chi-square test. The LC84, LC50, and LC16 points were read from the line, and the total number of animals used between the 16% and 84% points (N) were noted. Then the confidence limits of LC50 were found as follows:

$$f = \left(\tfrac{1}{2}\,(LC84/LC50 + LC50/LC16)\right) \exp\left(2.77 / N^{\frac{1}{2}}\right)$$

$$LC50 \times f = \text{upper limit}$$

$$LC50 / f = \text{lower limit}$$

Reproductive impairment of Daphnia (RI 50) was found in the same way, after expressing the number of offspring produced at each concentration as a percentage of the number produced in the control.

In the few cases when less than two partial kills occurred, LC50 values were calculated by the moving average method,[37] which does not require a straight line graph:

R_i = number of deaths at dosage i (= 1, 2, 3...L)

where 1 = lowest dosage
L = highest dosage

S_i = number of survivors at dosage i

N = number of individuals per tank

F = geometric factor between dosage levels (>1)

t = Student's t for (P = 0.05) and (N-1)(L-1) d.f.

$$G = \left((N(L-1)/2) - R_1 - R_2 - \ldots\ldots R_{L-1} \right) / (R_L - R_1)$$

$$M = \log D + \log F \left(\left((L-2) / 2 \right) + G \right)$$

$$V = \frac{1}{R_L - R_1} \left[\frac{(1-G)^2 R_1 S_1 + R_2 S_2 + \ldots\ldots + R_{L-1} S_{L-1} + G^2 R_L S_L}{N - 1} \right]^{\frac{1}{2}}$$

$$LC50 = \text{antilog}\,(M \pm t\,PV)$$

To determine differences in mean weight, length, etc., between experimental tanks, a one-way analysis of variance was used, provided the variances were homogeneous (Bartlett's Test). If a significant F value occurred, the differing tanks were found by Duncan's Multiple Range Test. These tests are described in most general statistics texts.

Routine water quality data such as temperature and oxygen were computed as monthly means for each tank of the chronic tests. All parathion analyses, taken each week, were used to calculate a mean, range, and standard deviation (SD) for each test tank.

SECTION V

RESULTS

BLUEGILL

Acute Bioassay

Five preliminary and one final acute bioassays were performed at 22°C. Table 4 gives the test results and calculated LC50 values for the final test. The sequence of symptoms of acute poisoning were: lethargy, dark discoloration, hypersensitivity, tremors, coughing, convulsions, extended pectoral fins and opercula, loss of equilibrium, scoliosis, hemorrhages, tetanus, and death.

Whole fish from the acute test were analyzed for parathion residues (Table 5). They had been exposed for various lengths of time depending on mortality rate. Figure 1 shows that the increase in parathion concentration in tissue was roughly linear.

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Table 4. BLUEGILL ACUTE TEST

Number Surviving Per Tank

Hour	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
0	5	5	5	5	5	5	5	5	5	5	5	5
24	5	5	3	2	4	5	4	3	5	3	3	4
48	5	5	0	0	4	3	3	3	5	3	3	4
96	5	5	0	0	1	1	2	1	5	2	3	2
144	5	5	0	0	1	1	2	0	5	2	3	2

Analyzed Parathion Concentration, mg/l

0	nd	nd	2.10	2.19	1.24	1.24	0.63	0.63	0.50	0.50	0.33	0.33
48	nd	nd	2.25	2.25	1.25	1.25	0.64	0.64	0.53	0.53	0.35	0.35

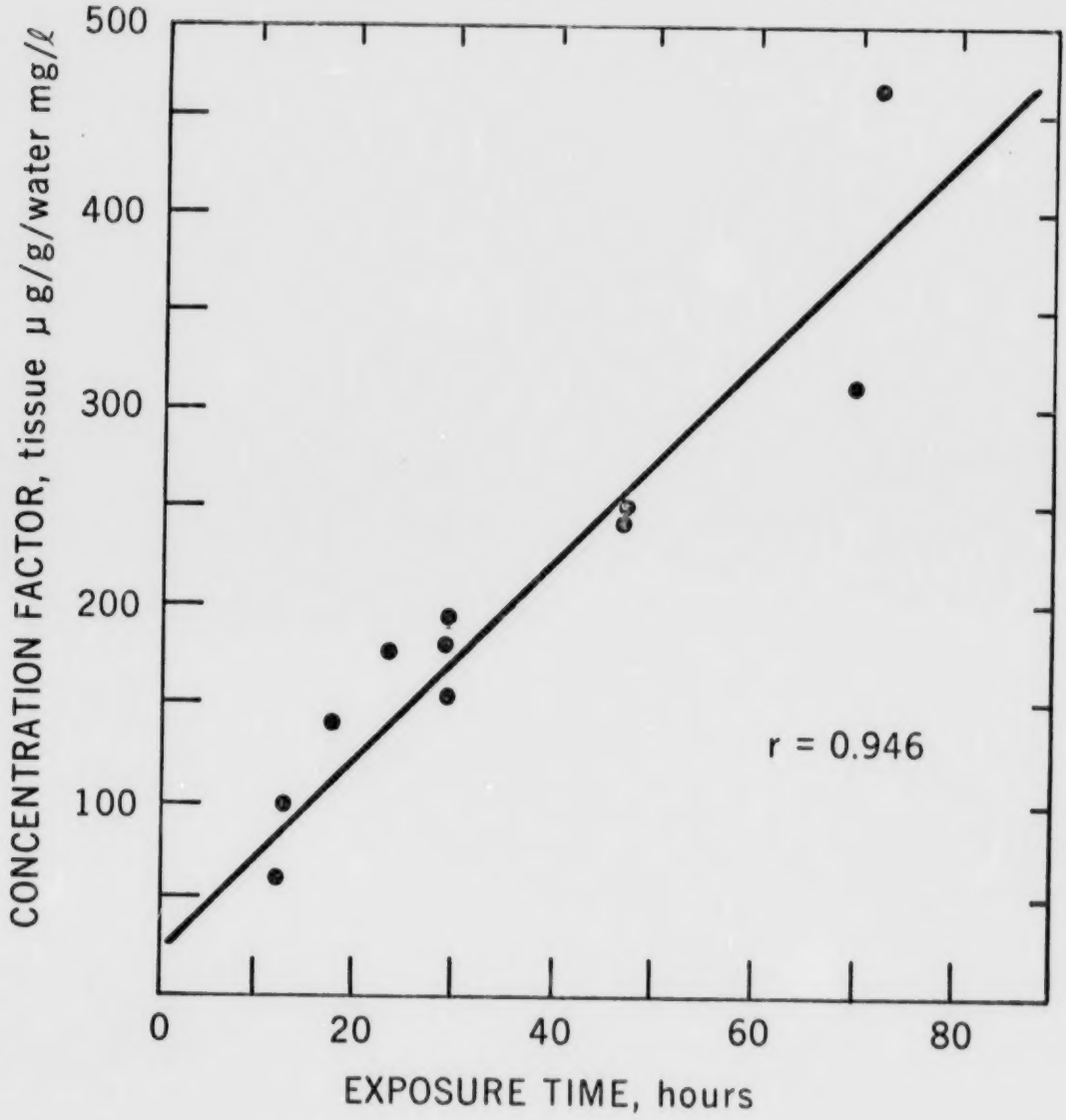
Median Lethal Concentrations, mg/l

Hour	LC50	Upper CL	Lower CL
24	2.25	4.35	1.16
48	1.15	1.92	0.68
96	0.51	0.72	0.36
144	0.46	0.95	0.22

Table 5. PARATHION RESIDUES IN WHOLE BLUEGILLS
ACUTE TEST

Tank	Parathion in water, mg/l	Parathion in fish, mg/kg	Exposure, hours	Concentration factor
5A	0.34	49.9	18	145
5B	0.34	61.4	24	173
4B	0.51	50.7	12	98
4B	0.51	32.5	12	63
3A	0.64	294.1	72	462
3B	0.64	197.8	70	311
2B	1.25	318.6	46	256
2B	1.25	311.6	46	250
1A	2.22	402.9	29	181
1A	2.22	430.0	29	193
1B	2.22	338.3	29	152

Figure 1. Concentration factors for parathion residues in whole bluegills at various exposure times.



Bluegill Chronic Test

The maximum no-ill-effect level of chronic parathion exposure to bluegills fell between 0.17 and 0.34 µg/l based on deformations of adults. Growth rate, spawning, and fry survival were not clearly affected by toxicant concentrations up to 3.2 µg/l.

A summary of parathion analyses in water, water quality, and test temperature are given in Tables A1, A2, and A3. Bluegill survival, (Table A4) showed that test concentrations did not cause significant mortalities. Some deaths of extremely deformed fish did occur. But the majority of deaths followed handling, particularly at the time of thinning. For this reason, weight measurements were not taken more frequently during the test. There were no significant differences in weights among tanks at the start of the test, at the thinning, or at the end. Final sizes of fish were approximately 17 cm, 105 g for females, and 21 cm, 200 g for males.

Within the first four months of testing, tremors, scoliosis, and extended pectoral fins were observed in the two highest concentrations. In six months, protrusions in the hyal or "throat" region were noticeable at these levels. The same symptoms continued, and gradually increased during the 23-month test. Table 6 lists the incidence of scoliosis and "throat" protrusions. The percentages are somewhat different at 18 and 23 months because some very deformed fish were removed at thinning.

A typical protrusion in the hyal region is shown in Figure 2. It was a semi-rigid, vascularized mass of fatty and connective tissue extending from the branchiostegal and urohyal bone area. Scoliosis or lateral bending of the spine, usually posterior to the dorsal fin, is illustrated in Figure 3. In bluegills, the bends were both right and left-handed.

Spawning proceeded successfully through the second summer. Egg production, hatchability, and fry survival are summarized in Table 7. Numbers per tank are listed chronologically downward. In general, the hatchabilities improved as we reduced handling of the egg cups. Likewise, 7-day fry survival improved as more Artemia were fed. Spawning data cannot be correlated to parathion level.

The numbers of males and females present were sufficient to provide good spawning:

Table 6. INCIDENCE OF DEFORMITIES - BLUEGILL CHRONIC TEST

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Mean parathion conc., µg/l	0.06	0.06	3.23	3.14	1.53	1.59	1.00	1.00	0.34	0.34	0.16	0.17
18 months												
Number of fish	10	15	17	13	20	17	16	15	9	13	10	9
Scoliosis, %	0	13	59	38	45	24	38	33	11	0	0	0
Throat, %	0	13	88	100	70	76	75	67	11	0	0	0
23 months												
Number of fish	9	9	8	8	11	9	9	9	8	9	7	7
Scoliosis, %	0	11	25	50	18	33	22	22	0	0	0	0
Throat, %	0	0	62	88	64	100	89	89	38	56	0	0

Figure 2. Male bluegill exposed to 1.5 µg/l parathion for 23 months. Length, 21 cm. Arrow indicates abnormal protrusion of fatty and connective tissue.

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Figure 3. Two adult bluegills exposed to 1.5 µg/l parathion for 23 months, showing scoliosis. Dorsal view.

Table 7. BLUEGILL CHRONIC EXPOSURE TO PARATHION - SPAWNING RECORDS

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Nominal parathion conc., µg/l	0	0	4.00	4.00	2.00	2.00	1.00	1.00	0.50	0.50	0.25	0.25
Hundreds of eggs spawned	5	49	20		1	90	38	105		271	26	229
	2		41		80	71	27	8		10	112	
	28		44		10	2	9	209		48	38	
	15		66		9	25	15	2				
			167		45	64	103	49				
								14				
Percent hatch, 3 days	72	46	44		49	40	80	37		57	83	86
	57		46		36	4	59	35		77	80	
	74		49		62	61	45	9		32	79	
	84		86		76	10	64	16				
			78			62	82	18				
								18				
Percent survival, 7 days, as % of hatch	92	56	66		68	78	0	68		32	100	22
	98		92		76	8	86	36		96	94	
	96		90		100	92	80	20		100	46	
	84		100		60	100	90	97				
			100			84	96	36				
								59				

Table 7. BLUEGILL CHRONIC EXPOSURE TO PARATHION - SPAWNING RECORDS (CONT.)

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Percent survival,[a] 14 days	8	10	0		0	0	0	0		0	32	2
	78		4		18	0	2	0		78	28	
	26		28		30	48	20	0		100	-	
	14		82		0	80	18	85				
						0	28	10				
								0				
Percent survival,[a] 21 days	6	10	-		-	-	-	-		-	28	0
	76		0		16	-	2	-		66	28	
	20		22		16	46	20	-		84	-	
	2		68		-	46	18	81				
			-			-	28	0				
								-				
Percent survival,[a] 30 days	6	8	-		-	-	-	-		-	24	-
	76		-		16	-	2	-		64	22	
	18		22		16	46	18	-		62	-	
	-		64		-	34	14	5				
			-			-	26	-				

[a] Calculated as percent of larvae hatched.

Tank	Males	Females	Tank	Males	Females
OA	2	7	3A	1	8
OB	1	8	3B	3	6
1A	4	4	4A	3	5
1B	4	4	4B	3	6
2A	3	8	5A	2	3
2B	3	6	5B	3	4

Growth rates of 30, 60, and 90-day-old bluegill fry were significantly different, but not related to toxicant. Mean lengths and survival, Table 8, correlated inversely with numbers per tank. Large quantities of food were given four times daily. It appears that the larval chambers were too small for 40 fry. Regardless of the starting number of fry, each tank population diminished to a few individuals at the end of the three months. At 90 days, one fish in tank 1A (3.4 $\mu g/l$) had developed scoliosis.

During the first two months of the chronic test fish at 8.0 $\mu g/l$ began to show tremors. When these were removed after 64 days, their brain AChE activity was 1.97 $\pm$ 0.40 μmol/min/g wet wt. This is about 11% of normal bluegill brain activity. Although the fish showed signs of poisoning, they were not moribund. Tables A5 and A6 show the extent to which normal AChE activity was inhibited in the chronically exposed fish. The slightly greater depression in Table A6 was probably caused by higher parathion levels in water during the month before termination (Table A1). The majority of fish in all tanks except the lowest and control appeared hypersensitive to stimuli. When startled, they swam away or towards the signal, almost randomly. A disturbance could trigger clonic (rhythmic) or alternating convulsions in some fish.

Parathion residues of bluegill tissue at 18 and 23 months exposure are reported in Tables 9 and 10. In muscle, the levels are no greater than 25 times the water concentration. Blood levels are similar or slightly higher. The unusually high levels in Tank 3 (see Table 10) were affected by accidentally high water concentrations for two weeks before the sampling. The greatest residues were in ovaries which is reasonable because of their high lipid content. Although the data are semi-quantitative, the residues in spawned eggs are similar to those in the ovaries.

Table 8. SURVIVAL AND LENGTHS OF BLUEGILL LARVAE

	30 day		60 day		90 day	
Tank	Number	Mean length, mm	Number	Mean length, mm	Number	Mean length mm
OA	40	10.1	3	19.7	2	23.0
OA	9	11.3	7	14.9	3	20.7
OB	4	11.0	3	14.0	3	23.7
1A	31	10.5	13	13.2	11	18.5
1A	11	13.2	10	19.7	8	22.8
2A	8	13.6	7	23.4	5	32.4
2A	8	15.1	8	18.2	7	24.9
2B	23	10.7	6	16.8	6	22.2
3A	8	13.2	7	20.3	6	28.7
3A	12	9.4	4	12.2	3	18.0
4B	32	9.1	7	13.6	3	26.3
4B	32	8.9	4	16.2	4	20.5
5A	12	10.5	11	16.3	7	23.1
5A	12	11.5	10	14.8	4	28.8

Table 9. PARATHION RESIDUES IN BLUEGILLS

18 MONTH EXPOSURE

(µg/kg wet wt)

Tank	0	5	4	3	2	1
Nom. parathion µg/l	0	0.25	0.50	1.00	2.00	4.00
Muscle residue	5.4	9.8	16.9	16.0	13.1	107.0
	27.8	9.6	6.6	14.2	16.1	92.4
	0.3		23.7	11.6	17.4	97.8
	2.9		7.1	13.4	10.1	90.1
	5.8			14.1	39.6	141.2
				13.5	39.9	128.4
				12.3		
Mean ± SD	8.4±11.0	9.7±0.1	14.6±10.0	13.6±1.4	22.7±13.4	109±20.8
Blood residue	29.9	15.2	17.6	35.8	41.4	163
		19.7	19.5	49.9	119.8	288
				27.0		
Ovary residue	17.8		23.9	74	144	3436
	21.2		30.6	214	158	
	31.2			238	643	
	31.0				585	
	20.7					
Egg residue	nil	-	73	552	179	813
	-	-	nil	246	134	1089
						429

Note:

Recovery of spiked muscle samples (equivalent to 100 µg/kg) gave 88.4% recovery in a single test.

Precision among triplicate samples gave a coeff. of variation of 13.1%

All blood residues except No. 1 and No. 2 sets should be considered semiquantitative since levels are at or near detectable minimum in the direct extraction samples. No evidence of paraoxon was found.

Table 10. PARATHION IN BLUEGILLS

23 MONTHS EXPOSURE

(μg/kg wet wt)

Tank	0	5	4	3	2	1
Nom. parathion, μg/l	0	0.25	0.50	1.00[b]	2.00	4.00
Muscle residue[a]	1.2	1.0	2.1	30.2	5.4	26.1
	0.9	3.7	1.2	2.3	14.6	12.1
	0.8	3.6	11.6	32.6	14.2	12.8
	0.2	1.2	6.0	1.1	8.3	31.2
	0.9	4.5	0.8	14.4	25.3	10.6
	1.1	3.1	4.4	23.7	4.7	10.9
Blood residue	0.9	0.6	6.8	253.0	37.5	133.7
	1.2	1.8	9.4	118.5	42.7	159.6
	2.1	1.5	8.1	62.9	56.0	125.3
	< 0.1	3.9	25.7	78.7	61.7	123.7
	< 0.1	3.8	3.7	104.3	51.0	131.4
	2.0	4.0	8.5	97.8	67.2	164.2
	< 0.1	4.1				157.3
	.	9.4				111.0

[a]These are all at or below reliably detectable levels (~25μg/kg). Precision at these low levels is estimated at about ±50%.

[b]Mean parathion concentration for last month of test was accidentally high: 2.5μg/l (Table A1).

BROOK TROUT

Acute Test

Two preliminary and one final acute test were completed at 12°C. The 96-hour LC50 for the final test was 1.76 mg/l (Table 11). During the test, no mortality occurred only at the highest concentration (Table A7). Many fish in the three highest concentrations showed symptoms of lethargy, irregular breathing, and light discoloration of the skin. No visible scoliosis, convulsions, or hemorrhages appeared. There was no evidence of disease during the acclimation period or test.

The 96-hour result was normalized for 5% mortality in the control by substituting 100 (R-5)/95 for each percent mortality, R. The calculated 48-hour value is higher than the 24-hour one although the mortality remained the same. This was a consequence of the higher parathion analyses at 48 hour than at the start (Table A7).

Residues in trout muscle taken from the same test fish are shown in Table 12. Because the trout were removed as they died, the exposure times for these fish are not uniform. The concentration factors illustrate the extent to which trout can concentrate parathion within a few days. Further results of trout uptake studies are reported in Appendix B.

Brook Trout Chronic Test

At parathion levels of up to 7 µg/l adult brook trout showed no deleterious effects over a nine month period. No significant differences in growth rate, maturity, or spawning success were judged to be caused by the parathion dosages. The brook trout exposed to these levels, however, showed uptake of relatively large parathion concentrations in blood, muscle, and other tissues.

Water quality parameters, temperature, and parathion analyses for the test are given in Tables A8, A9, and A10. Most of the mortalities which occurred during the test (Table A11) can be attributed to disease, either fungus or furunculosis, or to failures in the water delivery system. No fish developed symptoms typical of acute organophosphate poisoning such as scoliosis or convulsions. Weight measurements made at the start, at 4 months, and at 9 months (Table A12) indicate a uniform weight increase at all exposure levels. No mean weights were found to be significantly different at the beginning or end of the test. Most of the largest weight gains can be explained by lower numbers of fish in those tanks.

Table 11. BROOK TROUT ACUTE LC50

Hour	LC50, mg/l	Upper CL, mg/l	Lower CL, mg/l
24	2.25	2.58	1.96
48	2.57[a]	2.78[a]	2.38[a]
96	1.76	2.10	1.48

[a]Estimated because of change in parathion conc. See page 40.

Table 12. PARATHION RESIDUES IN BROOK TROUT MUSCLE

Acute Test

Exposure, hr	Mean water conc., mg/l	Muscle conc., mg/kg wet wt.[a]	Concentration factor
8	3.18	217	68
8	3.18	346	109
114	1.86	165	89
114	1.86	215	116
144	0.53	121	228
144	0.53	83	157
140	0.27	70	259
140	0.27	93	344

[a]each analysis from a separate fish

Fish discarded from the test at the time of thinning, six months, were analyzed for parathion residues in blood, muscle, and other tissues, as well as acetylcholinesterase activity. The blood residues, in Table 13, were plotted against the analyzed parathion levels in the water at the time of removal (Figure 4).

A least-squares line fitted to the data has the equation:

$$C_b = 207\ C_w - 39$$

where C_b is concentration in blood, and C_w the concentration in water. Thus the trout took up about 200 times as much parathion in the blood as was found in the water.

The residue levels for muscle and other tissues show large concentration factors as well (Table 14). The muscle residues were plotted against water concentration in Figure 5 and fitted to a curve by eye. If treated as a straight line, this has an equation of:

$$C_{fl} = 553\ C_w - 176$$

No paraoxon was detected in any of the analyses despite the use of solvents to extract it from the clean-up columns. The residues reported in Table 14 were the highest concentrations found in any chronic exposure. Brain acetylcholinesterase activities of the same fish, Table 15, were similarly plotted. Figure 6 shows that about 5.6 $\mu g/l$ of parathion was required to depress the brain AChE activity to 50% of normal. However, no gross observable symptoms of central nervous system poisoning were detected in the fish in the highest dosage.

Brook Trout Spawning

The brook trout spawned between Dec. 23 and Jan. 15, test dates. A summary of the number of spawnings, egg production, and survival of the embryos and larvae is given in Table 16. Only a very small percentage of embryos were deformed, and these did not correlate to parathion concentration. The uniformly low hatchability of the embryos is attributed to handling damage and high temperatures at the time of spawning. The fact that the test temperature (13°C) was greater than optimal (9°C) probably caused the small number of spawnings and delayed development of the fish[39]. As a check of maturation, the gonad weights of the discarded fish were taken at the time of thinning (Table A13), and at the test termination - after spawning (Table A 14). The gonad weights were divided by the fish's total weight to arrive at a gonad-osomatic index expressed as a percent. The weights shown

in Table A14 are somewhat biased since small and immature fish were selectively discarded. For both males and females, the development was uneven and did not appear to be related to toxicant concentration. The later gonadosomatic indexes show that spawning was not complete. For example, female gonadosomatic indexes greater than 1.00 represent loose eggs in the body cavity which were never released.

Adult Brook Trout Termination

The adult brook trout were terminated on February 6, three weeks after the last spawning. Blood was obtained from 34 fish representing all concentration groups and was analyzed for parathion residues. Muscle tissue from 31 fish was analyzed for parathion. Residue analyses for parathion were also done on kidney, gill, gonad and gastrointestinal tract samples from each of 2 to 3 fish in each exposure concentration group. Brain ACHase was determined on 39 fish.

Tissue residue results, including blood levels, are tabulated in Table 17. Blood levels versus water concentrations of parathion analyzed at the time of removal of the fish are plotted in Figure 7. Blood and tissue residue levels measured after 9 months exposure were lower than corresponding values determined on fish sacrificed after 6 months exposure.

Several of the adult trout from each parathion concentration groups were exposed to flowing water without toxicant for 48 and 168 hours. Blood and muscle parathion residue analyses on these fish are shown in Table 18. Brain ACHase determinations were also done and results are given in Table 18. ACHase inhibition was essentially irreversible over the time studied (168 hours). The blood showed a rapid loss of parathion within 48 hours and a subsequent slower decrease. Muscle parathion concentrations decreased more slowly than blood levels. The limited samples available and the large variance among individual fish make it difficult to quantify "wash-out" rates but the blood and tissue uptake appears to be readily reversible.

Some general conclusions may be drawn on the basis of the 6 and 9 month analyses. Parathion residues in blood correlate with water concentrations at the time of sampling; blood levels appear to rapidly reflect exposure levels in the water. Tissue residues also correlate with water concentrations, however tissue levels will change less rapidly with change in water concentrations. High correlation exists between blood parathion concentrations and tissue parathion concentrations when measured after chronic exposure (6 or 9 months); the correlation coefficient for muscle parathion versus blood parathion was 0.967 ($\rho < 0.001$).

The high variance in water concentrations measured on a weekly basis probably account for the differences in residue results observed at 6 and 9 months. Parathion appears to be reversibly taken-up and released and residue levels reflect the immediate past exposure conditions.

Brain AChase levels, as expected, exhibited an inverse dependence on water concentration. The degree of inhibition was less marked than with bluegills. Essentially the same AChase effects were found at 6 and at 9 months. Relatively high variance within the concentration groups and especially the control group was found. However the highest concentration group exhibited AChase levels significantly lower than the other experimental groups (excluding the control group) ($\rho = 0.05$).

Nineteen-day-old brook trout eggs were incubated at 10°C in three concentrations of parathion and without parathion until hatched. The following percent survivals to hatch were observed:

control	10 µg/l	32 µg/l	100 µg/l
77.5%	52.5%	41.5%	38.4%

Eggs in the 32 and 100 µg /l solutions exhibited poor development and more transparent chorions than controls. Some of the eggs in both concentrations showed cerebral development without corresponding spinal development. Part of the lower embryo survival at the two highest levels resulted from premature hatching. Although the hatchability at 10 µg/l was less than the control group, development appeared to be normal.

Table 13. PARATHION IN BROOK TROUT BLOOD

Six Month Exposure, Individual Fish

(µg/l)

Tank	OA	1A	2B	3A	4A	4B	5A	5B
	6.2	1007	737	647	113	153	68	79
	63.7	1597	885	695	96	151	24	58
	10.6		1139	726	96		61	69
	40.5		776	337	93		52	103
	0.5		662	515			101	80
	6.9		528	654			42	50
				649			68	110
							50	
Mean	21	1302	788	603	100	152	58	78
SD	25	417	208	136	9	1	23	24

Figure 4. Parathion in brook trout blood vs. parathion in water, six month exposure.

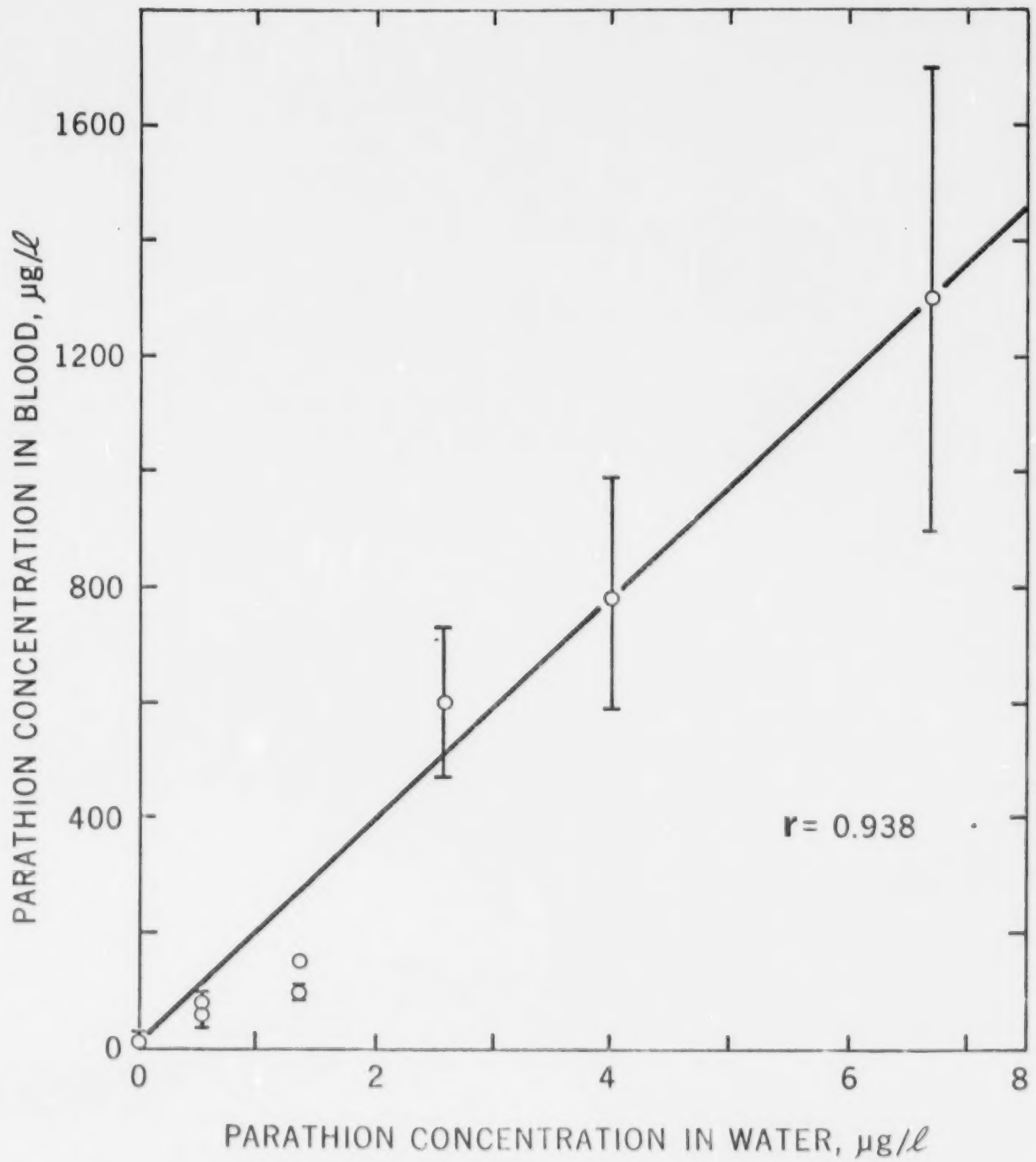

Table 14. PARATHION RESIDUES IN BROOK TROUT TISSUE

Six Month Exposure

(μg/kg wet wt)

Tank	OA	1A	2B	3A	4B	5A	5B
Water conc. μg/l	0.03	6.7	4.0	2.6	1.4	0.6	0.6
Muscle	nil	4454[a]	1958	1258[b]	176[a]	244	171[a]
	18	3229[a]	1820[a]	1411[b]	474[a]	130	139
	12		1874	921	606		
	nil			998			
				1124			
Kidney	nil	2942	998	1245	123		nil
	125	556	815	1051	nil		277
			3332				
Gill	nil	5954	2503	1932	492	-	nil
	26	4090	2430	2659	516		160
			3294				
Gonad	nil	2208	2136	2050	175		nil
	16	1344	414	1284	11		176
			579				

[a]Average of duplicate samples
[b]Average of triplicate samples
All others are single samples

Reagent Blank = 0.3μg/kg
Recovery % at 1000 μg/kg level averaged 90.8±7.6% (coeff. variation = 8.4%); range 83.1 to 103% for seven tests.
All values uncorrected for % recovery.

Figure 5. Parathion in brook trout muscle vs. parathion in water, six month exposure .

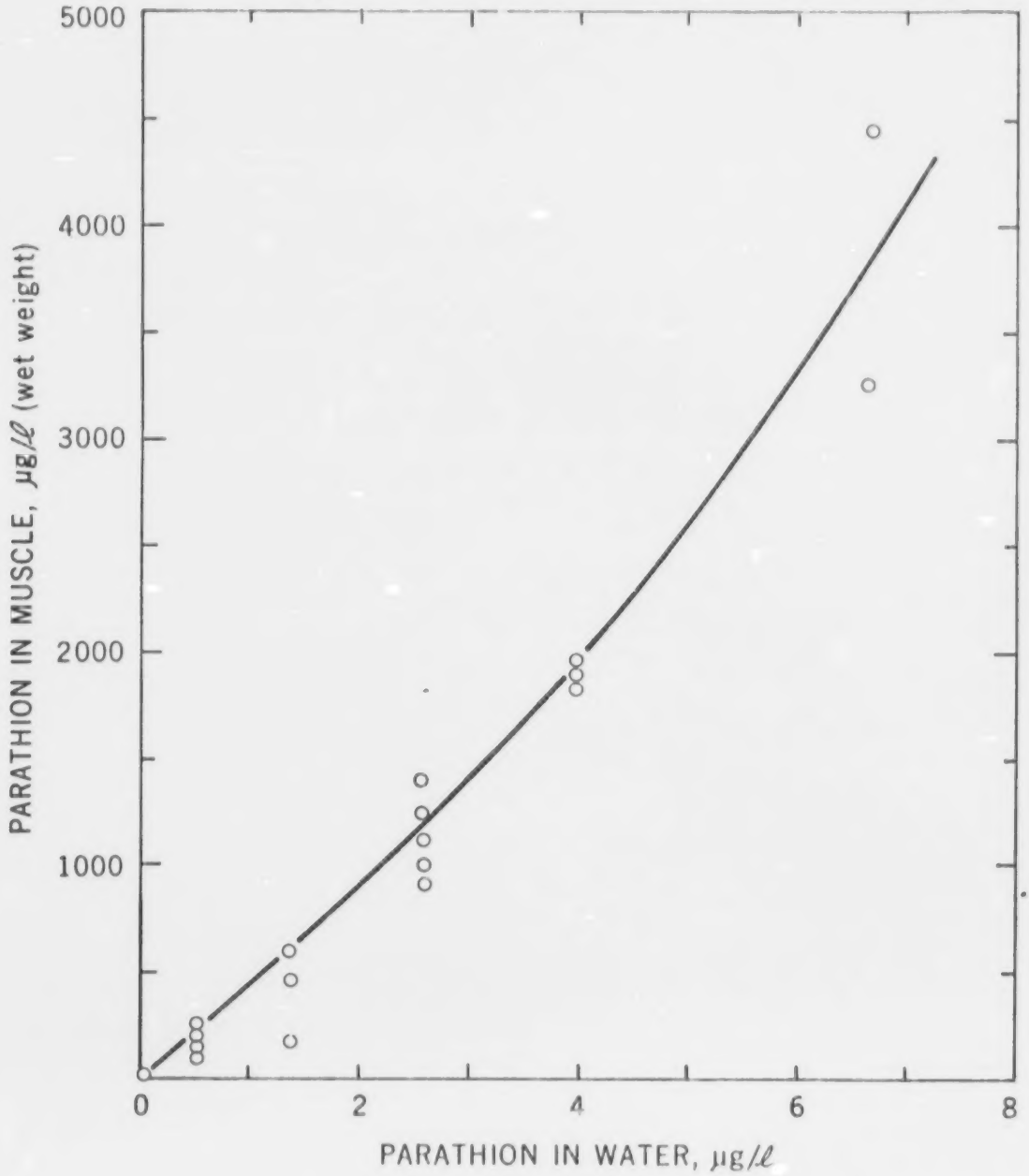

Table 15. BRAIN ACETYLCHOLINESTERASE ACTIVITY OF BROOK TROUT

Six Month Exposure, Individual Fish

(μ mol/min/g)

Tank	OA	1A	2B	3A	4A	4B	5A	5B
	6.60	3.79	5.22	4.19	4.96	4.28	5.51	6.72
	6.49	1.88	3.44	4.42	4.65	4.59	4.96	6.49
	6.63		5.34		5.85		5.85	
	6.66		4.16		6.03		6.72	
	5.85						5.74	
	6.57						6.49	
							4.71	
Mean	6.47	2.84	4.54	4.30	5.37	4.44	5.71	6.60
SD	0.31	1.35	0.90	0.16	0.67	0.29	0.74	0.16

Figure 6. Brook trout brain acetylcholinesterase activity vs. parathion concentration in water, six month exposure.

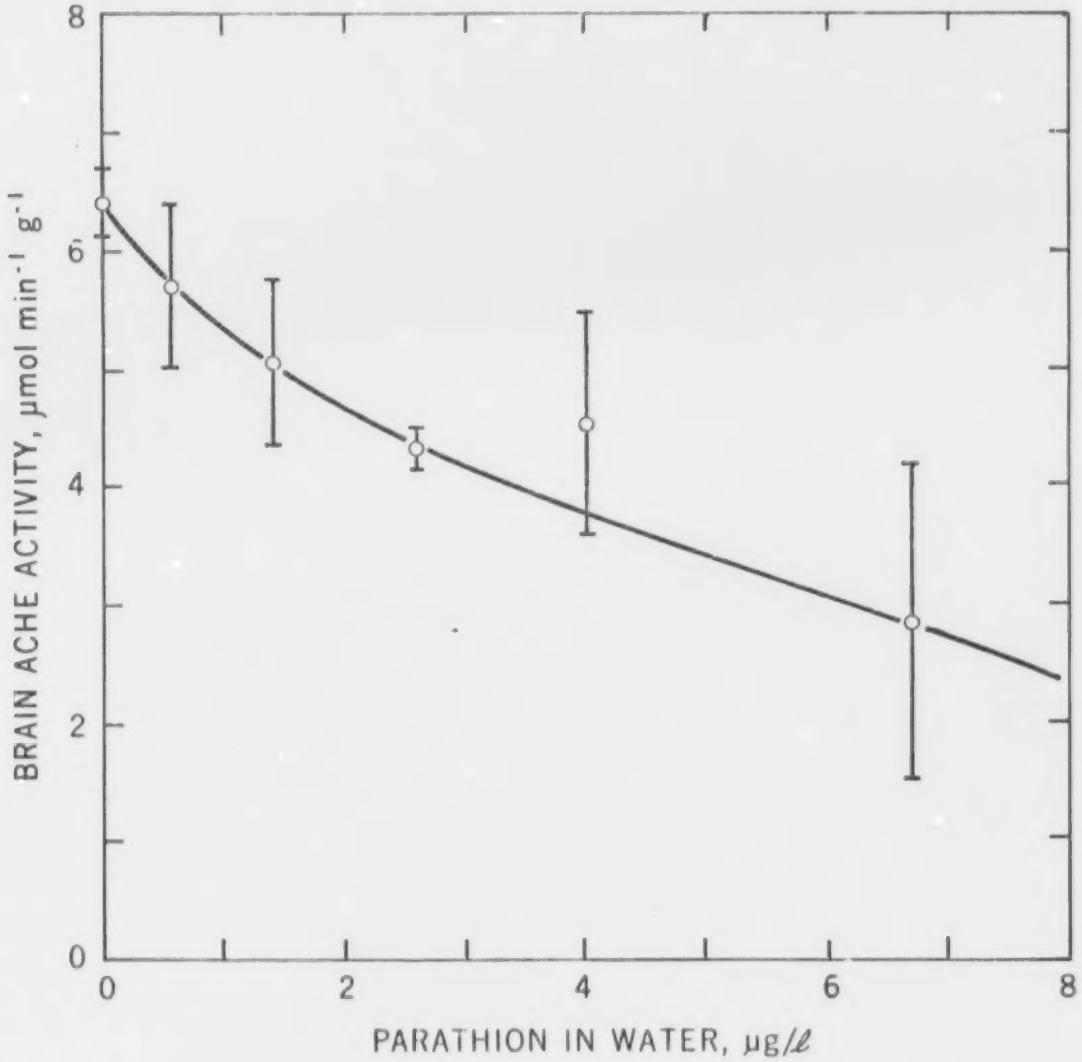

Table 16. BROOK TROUT SPAWNING RECORD

Tank	OA	1B	2B	3A	3B	4A	4B	5A	5B
Number spawnings	1	2	3	2	2	2	3	7	1
Total eggs spawned	360	1249	787	716	924	929	1607	479	48
12 day viability, % of spawn	26	85 42	2	76 56	15	100	78 0	24	
Total hatchability, % of spawn	6	12	20	12	0	6	22	4	0
30 day fry survival, % of hatch	0	0	10	67	0	67	18	0	0

Table 17. BROOK TROUT PARATHION RESIDUES AND AChE - 9 MONTHS

Tank	Parathion in water µg/l	Blood	Muscle	Gill	Kidney	Gonad	G. I. Tract	AChE, µ mol/min/g
OA	0.01	23	0.8					3.97
		42	0.6	3.1	0.2	<0.01	0.7	5.04
		94	1.9					5.12
		23	1.0					3.01
OB	0.01	58	0.6	1.4	0.1	-	28	4.49
		33						5.77
		29	0.1					4.62
		20						4.19
1A	8.30	733	1,220	3,172	400	1,812	8,989	3.44
		1,021	399					2.79
		990	4,157[b]					2.15
1B	8.72	625	1,623					3.17
		743	876	1,727	217	939	160	4.39
		720	2,181[a]	1,143	614	1,780	11,598	3.86
2A	5.53	361	297					6.24
		355						4.45
		-	671	2,358	311	1,054	5,666[c]	5.91
2B	4.24	337	318	1,483	374	705	200	4.46
		446	-					5.21
		304	-					6.17
		-	413[a]	1,085	78	519	352	4.71

Table 17. BROOK TROUT PARATHION RESIDUES AND AChE - 9 MONTHS (CONT.)

Tank	Parathion in water µg/l	Blood	Muscle	Gill	Kidney	Gonad	G. I. Tract	AChE, µmol/min/g
3A	2.86	184	270					4.30
		252	189[a]	703	192	6	1,672	4.81
		482						4.35
		166	319[a]					5.44
3B	2.76	232[a]	272	959	183	407	1,105	4.42
		217[a]	511[c]	1,083[c]	774	15,048[c]	1,210	4.76
4A	1.45	114	7	179	11	36[c]	106	6.24
		111	118					6.06
4B	1.26	141						5.85
		87	44	265	53	-	110	6.00
		82	34					4.36
5A	0.53	41	33	138	16	69	135[c]	5.63
		40	58[a]					5.73
		52						5.27
5B	0.44	39	36					6.51
		33						5.65
		36	73	96	63[c]	38	28	5.56
		63						5.45

[a] duplicate samples average.
[b] analysis done immediately after fish was sacrificed; all other samples were stored at - 18°C for 2-1/2 months.
[c] probable interference contribution to parathion value

Figure 7. Parathion in brook trout blood vs. parathion in water, nine month exposure.

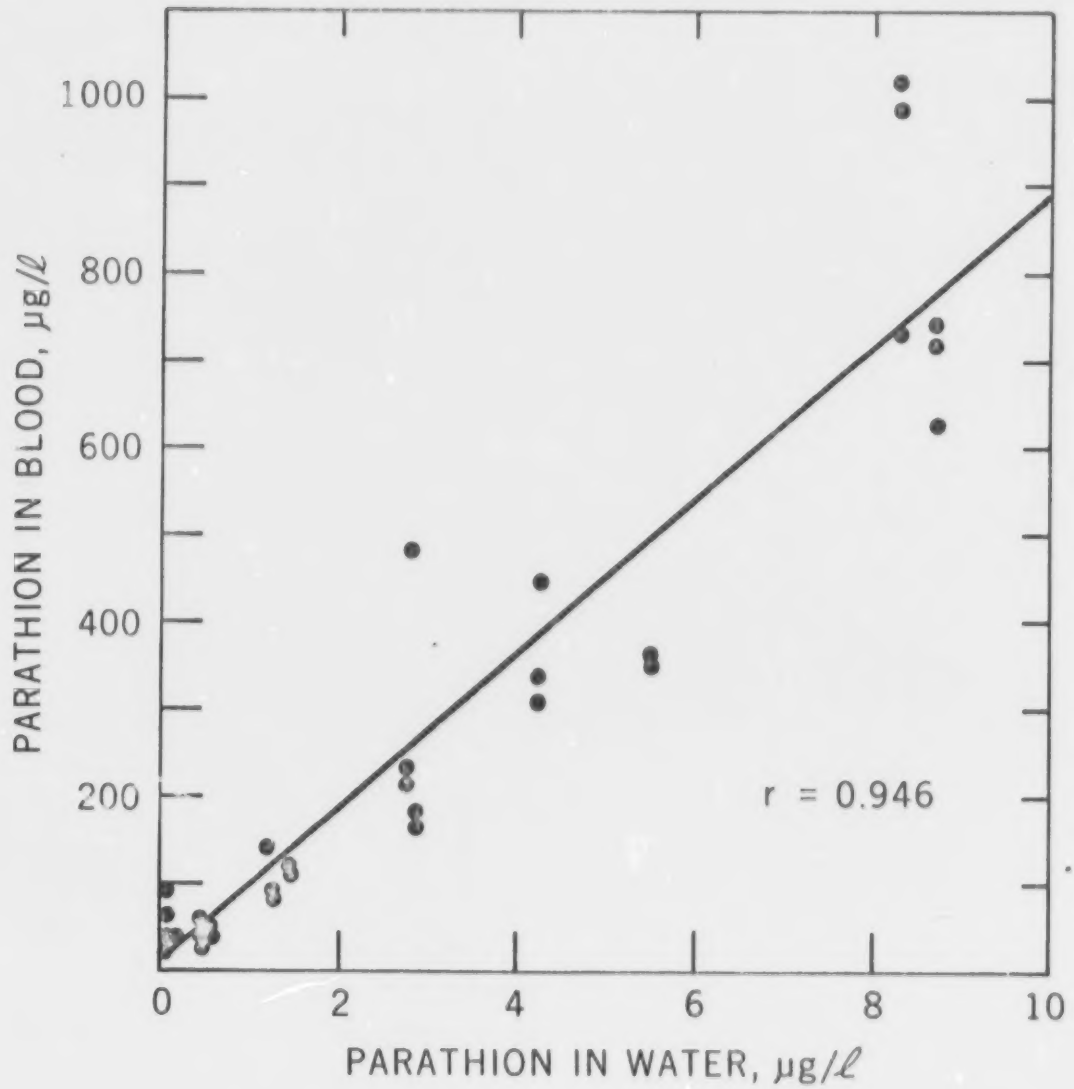

Table 18. BROOK TROUT PARATHION RESIDUES AND AChE - RECOVERY

48 HOUR ELUTION IN FRESH WATER

Tank	Blood residue, μ g/l	Muscle residue, μ g/kg	AChE μ mol/min/g
OA	27	35	5.42
OB	25	41	4.99
1A	507	587	4.45
1B	396	634	4.08
2A	223	478	4.09
2B	229	161	4.00
3A	182	187	4.48
3B	364	290	4.30
4A	94	92	5.77
4B	144	74	8.48
5A	123	85	6.11
5B	57	75	3.84

168 HOUR ELUTION IN FRESH WATER

Tank	Blood residue, μ g/l	Muscle residue, μg/kg	AChE, μ mol/min/g
OA	-	17	4.31
OB	19	-	5.74
1A	293	942	4.78
1B	422	1,124	3.62
2A	196	365	4.91
2B	127	462	
3A	65	223	4.23
3B	40	58	5.41
4A	35	60	4.59
4B	84	186	4.02
5A	21	62	9.08
5B	23	128	4.88

FATHEAD MINNOW

Acute Test

One preliminary static test using adult minnows at 22°C gave the following results based on measured initial concentrations:

LC50 - 24 hr = 2.28 (2.92 - 1.77) mg/l

48 hr = 2.02 (2.64 - 1.55) mg/l

96 hr = 1.60 (2.05 - 1.25) mg/l

A final flow-through test at 24°C using adults (approx. 2.2 g each) gave the results reported in Table 19.

Fathead Minnow Chronic Exposure

The fathead minnow chronic exposure gave a maximum acceptable parathion concentration of about 4 µg/l based on adult deformities and reproductive impairment. Water quality, temperature, and parathion levels for the test are summarized in Tables A16, A17, and A18.

For the first 30 days of the test, minnow larvae showed good survival and growth (Table A19). There were no significant weight differences between tanks. At 60 days, the mean length in tank 3B was significantly less ($P = 0.05$) than tanks 3A, 5A, and 5B, but not different from any others (Table A19). During the third month of exposure, symptoms of poisoning began to appear in several of the higher dosages. Convulsions and scoliosis were observed occasionally. By the time of spawning, there were deformities (including scoliosis, lordosis, and abnormally shaped heads) in almost half of the fish in the 50 µg/l tanks (Table 20). Weights, however, were not significantly different between tanks, as calculated by analysis of variance.

Spawning occurred first in the controls, and in the other tanks several days later. Egg production (Table 21) and the number of spawns per female were highest in the two control tanks. The hatchability rate did not appear to be related to parathion; the 30-day larval growth and survival were too sparse to judge toxicant effect.

Some whole minnows were analyzed for parathion during the test but because of their small size, the fish were pooled. Fish taken at 70 days exposure (the thinning), had lower residue levels than fish sampled later (Table 22). The maximum concentration factors for the chronically exposed minnows were about 100 to 170, which was less than trout, but greater than bluegills.

Table 19. FATHEAD MINNOW ACUTE TEST

24°C

Number Surviving

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B
Start	10	10	10	10	10	10	10	10	10	10
24 hr	10	10	0	0	0	0	9	6	9	8
48 hr	10	10	0	0	0	0	7	1	6	4
96 hr	10	10	0	0	0	0	6	1	6	4
Parathion[a], mg/l	nil	nil	5.31	5.88	1.92	2.24	1.04	1.04	0.53	0.49

[a] Means of four analyses.

Hour	LC50 mg/l	Upper CL, mg/l	Lower CL, mg/l
24	2.20	3.90	1.24
48	0.50	1.03	0.24
96	0.50	1.03	0.24

Table 20. MINNOW ADULT WEIGHTS AND DEFORMITIES - 8.5 MONTHS

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
No. females	8	9	11	9	7	5	6	8	9	7	8	5
Mean wt, g	1.15	0.98	1.05	1.16	1.29	1.32	1.25	1.07	0.94	1.09	1.22	1.23
No. males	4	4	2	2	3	2	2	4	4	1	5	8
Mean wt, g	2.30	1.88	1.92	1.38	1.78	1.46	1.88	1.99	1.73	1.75	1.99	1.90
% deformed	0.0	0.0	46.2	45.4	30.0	28.6	0.0	50.0	15.4	25.0	7.7	0.0

Table 21. MINNOW SPAWNING RECORD

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Total eggs spawned	2096	3591	463	51	251	979	103	397	2205	0	570	399
Number of spawnings	14	21	8	1	2	7	3	5	12	0	5	4
Spawns per female	1.75	2.33	0.73	0.11	0.29	1.40	0.50	0.62	1.33	0.00	0.62	0.80
Mean % hatch	72.4	88.2	65.5[a]	42.0	-	44.4	-	51.2	74.3	-	75.0	65.5
Spawns hatched	9	19	3	2	0	5	0	6	14	0	6	4
30 day survival, percent	70.0	42.5		40.0								12.5
Mean length, cm	0.98	1.49		1.20								1.68

[a] Four sets of eggs spawned in OB were hatched in 1A - 70% mean hatchability

Table 22. PARATHION RESIDUES IN WHOLE FATHEAD MINNOWS

(μg/kg)

Water parathion conc. μg/l	0.15	49.0	21.7	15.5	9.0	4.2
70 days exposure	-	1,040 (20)	615 (2)	251 (19)	214 (22)	256 (8)
82-138 days exposure	-	9,975 (3)	4,400 (5)	2,256 (3)	1,796 (1)	1,411 (4)
260 days exposure	14 (2)	8,300 (2)	2,270 (2)	510 (2)	601 (2)	846 (2)

Numbers in parentheses are numbers of fish pooled

DAPHNIA MAGNA

The acute and chronic toxicities of parathion to Daphnia magna are summarized in Table 23. Representative test data are in Tables A20 and A21. The daphnids responded to parathion poisoning within narrow limits. Thus, the MATC of 0.08 µg/l was very near the 3 week chronic LC50 of 0.14 µg/l.

The concentration needed to reduce reproduction by 50%, RI50, was greater than the chronic LC50. This occurred because young were produced during the third week of exposure at concentrations that later killed the adults (Table A21.)

Symptoms of toxicity during the acute exposures were erratic swimming in a circular motion, and later, inactivity and immobility.

The MATC of 0.08 µg/l (Table A21) was 0.08 times the 48-hour LC50 of 1.00 and 0.13 times the 96-hour acute value. The MATC was considered to be the highest concentration tested in which the number of young produced did not differ significantly from controls, as determined by analysis of variance.

For residue analyses, 0.4 to 20 mg collections of treated Daphnia were filtered and air dried. Hexane extracts were cleaned with a silicic acid column and analyzed by GC. No evidence of significant parathion residues could be detected above the GC background noise for any sublethal exposure levels. There is a possibility of decomposition of parathion during the air drying process.

Table 23, PARATHION TOXICITY TO DAPHNIA MAGNA

Acute LC50, µg/l

24 hr	48 hr	96 hr
2.70 (5.61 - 1.30)	1.00 (1.73 - 0.58)	0.62 (0.90-0.43)
3.21 (5.10 - 2.02)[a]	1.27 (1.63 - 1.09)[a]	0.65 (0.88-0.48)[a]

Chronic LC50, µg/l

1 week	2 week	3 week	RI50
0.28 (0.29 - 0.26)	0.25 (0.22 - 0.28)	0.14 (0.16 - 0.13)	0.24 (0.32 - 0.17)
0.56 (0.85 - 0.37)	0.38 (0.70 - 0.21)	0.19 (0.30 - 0.12)	0.48 (0.87 - 0.26)
0.35 (0.47 - 0.26)			
0.37 (0.74 - 0.18)			
0.45 (0.55 - 0.37)			

MATC = 0.08 µg/l

[a]Static test at 22°C
All other tests flow-through at 18± 1°C

GAMMARUS FASCIATUS

Results of the parathion acute and chronic tests to G. fasciatus are reported in Table 24. Two long-term tests were terminated when survival in the lowest concentration became less than 50% of the survival in the controls. Table A23 shows one of these chronic tests in which the controls and low levels began reproducing.

Table 24. PARATHION TOXICITY TO GAMMARUS FASCIATUS

LC50, µg/l

24 hr	48 hr	96 hr	Temp., °C
2.1 (3.8 - 1.2)	0.62	0.43 (0.65 - 0.29)	20
1.27 (1.62 - 0 99)	0.82 (0.99 - 0.68)	0.62 (1.16 - 0.33)	19
2.3	0.86 (1.23 - 0.60)	0.26 (1.00 - 0.07)	20
-	-	0.25 (0.29 - 0.21)	20
35 days - 0.09 (0.69 - 0.01)			19
43 days - 0.07 (0.50 - 0.01)			20

MATC <0.04 µg/l

CHIRONOMUS TENTANS

The C. tentans results summarized in Table 25 show a striking change in the LC50 with length of exposure. Between acute and chronic periods the lethal concentration varied over three orders of magnitude. Preliminary static acute tests using the tissue paper substrate were discarded when we discovered that parathion was removed from the water in several days. The flow-through tests eliminated this problem of decreasing water concentration.

Table 25. PARATHION TOXICITY TO C° TENTANS

Exposure, days	LC50, µg/l	Instar at start
1	660 (1040 - 420)	4
2	135 (703 - 26)	4
4	31.0 (43.4 - 22.1)	4
5	7.3 (11.8 - 4.6)	2
8	2.2 (3.2 - 1.5)	2
14	2.3 (3.4 - 1.6)	2
14	2.9 (3.7 - 2.3)	2

SECTION VI

DISCUSSION

Of the fish species tested, bluegills were most sensitive to parathion poisoning. The 96-hour LC50 of 0.51 (0.72 - 0.36) mg/l found for juveniles agrees with the 0.58 ppm value reported by Pickering, Henderson, and Lemke[40] for large bluegills in soft water. They also reported a much lower level, 0.095 ppm, for small bluegills under static conditions.

Yearling brook trout exhibited a 96-hour LC50 of 1.76 (2.10 - 1.48) mg/l. This is somewhat greater than the 72-hour LC50 of 0.92 mg/l given by Leland[16] for rainbow trout. Trout responded over a wider range of parathion levels than either bluegill or minnow.

Adult fathead minnows were killed by concentrations within the range of bluegill and trout response. A static test gave a 96-hour LC50 of 1.60 (2.05 - 1.25) mg/l while a flow-through test gave 0.50 (1.03 - 0.24) mg/l in the same time period. The static result agrees with literature LC50 96-hour values: 1.3 mg/l[40], 1.4 mg/l[41,42] and 1.6 mg/l[43]. By comparison, the flow-through result seems low. Test results were confirmed in preliminary testing, fish were handled carefully to avoid stress, and parathion concentrations were checked by independent analysts.

Chronic effects to bluegills, including scoliosis, abnormal protrusion of tissue, and depressed acetylcholinesterase activity, were found at parathion concentrations above 0.17 to 0.34 µg/l. Scoliosis was found under similar conditions to involve calcium deposition, and fusion of two to four vertebrae (Carter[14]). The protrusion in the "throat" area of many test fish was unexpected. Affected fish had shallow, rapid breathing and flaired gill covers - presumably because of nerve damage. The protrusion may result from mechanical damage to the branchiostegal area indirectly caused by spasmodic buccal pumping. Further histological characterization would help to determine the mechanism of such parathion-related tissue damage.

At 3.2 µg/l parathion, bluegills were able to survive with 16% of normal AChE activity. In a tank under optimum conditions and little disturbance, they could function, marginally. In nature, their ability to feed and escape predators would be doubtful.

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Brook trout exposed to 7.2 µg/l parathion for 9 months developed no harmful effects, as judged by weight gain, external appearance, AChE activity, and production of embryos. The data are not conclusive, however, about the sensitivity of the embryos and larval stages. A level of 32 µg/l reduced hatchability of embryos from untreated parents, while a level of 10 µg/l did not.

Fathead minnows showed deformities and reduced egg production in chronic parathion concentrations above 4 µg/l. No reduction in growth rate, hatchability or fry survival were demonstrated at the higher levels. Thus in both the bluegill and minnow tests, the juvenile and adult stages seemed more sensitive to parathion than their eggs or larvae. This could simply be a function of length of exposure. However, Lewallen[43] found that 2.0 mg/l parathion had no effect on one-week-old rainbow trout sac fry, while 70% of one-month-old fry were killed at the same level in 24 hours. Because parathion toxicity depends on activation, it is possible that older animals metabolize parathion more efficiently than young ones.

The retention of parathion in blood, muscle, and other tissues was demonstrated for all three fish species. Bluegill and trout showed concentration factors of several hundred in acute tests. In chronic tests, where residues probably reach a steady state, brook trout had the highest parathion in tissues, including muscle with 553 times the level in water. Whole minnows had concentration factors of about 200, while bluegills had, at the most, about 25 times the level in water. This suggests that bluegills metabolize parathion more readily than the other two fish, which could explain their greater sensitivity. We cannot rule out the possibility that this is a temperature as well as species effect. Test temperatures increased from trout, to minnow, to bluegill. It is possible that bluegills held at lower temperatures would metabolize less, and store more parathion. Further metabolic pathway studies in fish or in other cold-blooded animals should relate effects of temperature, rate of metabolism, and proportions of major metabolites.

The two crustaceans tested, D. magna and G. fasciatus, were more sensitive to parathion than either the insect, C. tentans, or the fish. For Daphnia, the maximum acceptable chronic level, about 0.08 µg/l was 0.13 times the acute level of 0.63 µg/l. The acute value is close to 0.8 µg/l reported by Priester[44] (50 hours) and Boyd[45] (96 hr.). Gammarus had a chronic effect level of much less than 0.04 µg/l - a lethal level in one month. At such low effect levels (which approach the chromatographic background for routine work) it became impractical to carry out meaningful tests for more than one month. Since crustaceans such as Daphnia and Gammarus are typical foods for many small fish, parathion levels in natural waters should be limited to less than 1 µg/l. In terms of protecting food chain organisms, and hence fish, this would not be an overly conservative level.

The midge, C. tentans, had a wide range of sensitivities to parathion, depending on flow rate, larval stage, and exposure time. It gave a 96-hour LC50 of 31 µg/l and chronic effect level of less than 3 µg/l, a lethal concentration in 2 weeks. The midge was the only species tested that is closely associated with sediment. The adsorption properties of parathion on the organic material used in the tests was probably the dominant influence on toxicity. Certainly the midge larvae could tolerate a relatively high single dose of parathion if it were adsorbed rapidly by the substrate.

A few problems stand out in the methods for chronic toxicity testing of aquatic species. For seasonally applied pesticides, a one or two year chronic test of constant concentrations in water may not be realistic. Only the shorter-lived invertebrates would normally be exposed for chronic time periods. Bluegills are not practical species for chronic tests unless a method is devised to insure spawning during the first summer in the laboratory. For all fish, egg handling, incubation, and larvae-rearing techniques used were cumbersome and required great labor. In many cases the harmful effects of handling outweighted the effect of the toxicant under study. Among the invertebrates, Daphnia and Chironomus proved to be convenient test organisms. In long-term tests, Gammarus had too poor survival using present culture techniques.

Finally, before accepting the results of such tests at face value, further understanding is needed of the pesticide in a natural system. Mechanisms of direct uptake and trophic concentration should be considered, as well as microbial degradation, and adsorption on sediments. The effect on fish productivity of a reduction of invertebrates by pesticides should be examined.

SECTION VII

REFERENCES

1. Lawless, E. W., R. von Rumker, and T. L. Ferguson. The Pollution Potential in Pesticide Manufacturing. EPA Tech Series Report. TS-00-72-04. 1972. 250 p.

2. Lichtenstein, E.P. and K.R. Schulz. Effects of Moisture and Microorganisms on the Persistence and Metabolism of Some Organophosphorus insecticides in Soils, with Special Emphasis on Parathion. J Econ Entomol 57:618-627. 1954.

3. Wolfe, H. R., D. C. Staiff, J. F. Armstrong, and S. W. Comer. Persistence of Parathion in Soil. Bull Envir Contam Toxicol 10:1-9. 1972.

4. Stewart, D. K. R., D. Chisholm, and M. T. H. Ragab. Long Term Persistence of Parathion in Soil. Nature. 229:47. 1971.

5. Gomaa, H. M., and S. D. Faust. Chemical Hydrolysis and Oxidation of Parathion and Paraoxon in Aquatic Environments, in Fate of Organic Pesticides in the Aquatic Environment, Washington, D. C., American Chemical Society. 1972. p. 189-209.

6. Eichelberger, J. W., and J. J. Lichtenberg. Persistence of Pesticides in River Water. Environ Sci and Tech. 5:541-544. 1971.

7. Graetz, D. A., G. Chesters, T. C. Daniel, L. W. Newland, and G. B. Lee. Parathion Degradation in Lake Sediments. J Water Poll Contr Fed. 2 (pt. 2):R76-R94. 1970.

8. Nicholson, H. P., et al. Insecticide Contamination in a Farm Pond. Trans Am Fish Soc. 91:213-222. 1962.

9. Miller, C. W., B. M. Zuckerman, and A. J. Charig. Water Translocation of Diazinon-C^{14} and Parathion-S^{35} off a Model Cranberry Bog. Trans Am Fish Soc. 95:345-349. 1966.

10. Baker, R. A. Pesticide Usage and its Impact on the Southeast. EPA Pesticide Study Series 8. 1972. 411 p.

11. O'Brien, R. D. Insecticides: Action and Metabolism. New York, Academic Press, 1967. p. 32-82.

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12. Weiss, C. M. The Determination of Cholinesterase in the Brain Tissue of Three Species of Freshwater Fish and its Inactivation in Vivo. Ecology. 39:194-199. 1958.

13. _____, Physiological Effect of Organic Phosphorus Insecticides on Several Species of Fish. Trans Am Fish Soc. 90:143-152. 1961.

14. Carter, F. L. In Vivo Studies of the Brain Acetylcholinesterase Inhibition of Organophosphate and Carbamate Insecticides in Fish. Ph.D. Thesis, Louisiana State U. 202p. 1970.

15. Hitchcock, M., and S. D. Murphy. Activation of Parathion and Guthion by Mammalian, Avian, and Piscine Liver Homogenates and Cell Fractions. Toxicol Appl Pharmacol. 19:37-45. 1971.

16. Leland, H. V., II, Biochemical Factors Affecting Toxicity of Parathion and Selected Analogs to Fishes. Ph.D. Thesis, U. of Michigan. 123p. 1968.

17. Potter, J. L., and R. D. O'Brien. Parathion Activation by Livers of Aquatic and Terrestrial Vertebrates. Science. 144:55-56. 1964.

18. Carlson, G. P., Comparison of the Metabolism of Parathion by Lobsters and Rats. Bull Environ Contam Toxicol. 9:296-300. 1973.

19. Nakatsugawa, T., N.M. Tolman, and P. A. Dahm. Degradation of Parathion in the Rat. Biochem Pharmacol. 18:1103-1114. 1969.

20. Mount, D. I., and H. W. Boyle, Parathion - Use of Blood Concentration to Diagnose Mortality of Fish. Environ Sci and Technol. 3: 1183-1185. 1969.

21. Mulla, M. S., J. O. Keith, and F. A. Gunther. J Econ Entomol. 59: 1085-1090. 1966.

22. Solon, J. M., J. L. Lincer, and J. H. Nair III. Effect of Sublethal Concentrations of LAS on the Acute Toxicity of Various Insecticides to the Fathead Minnow. Water Res. 3: 767-755. 1969.

23. Lowe, J. I., P. D. Wilson, and B. Davison. Laboratory Bioassays. Prog Rept. Bur Comm Fish Center for Estuarine and Menhaden Res. Contr. No. 98. Circ 335. p. 20-22. 1970.

24. Kemp, H. T., J. P. Abrams, and R. C. Overbeck. Effect of Chemicals on Aquatic Life. Water Quality Criteria Data Book, Vol. 3, 528 p. May, 1971.

25. Matsue, Y., T. Endo, and K Tabata. Effect of Parathion on Aquatic Animals in Sublethal Concentrations. Nippon Suisangaku Kaishi (Tokyo). 23:358-362. 1957-1958.

26. Davis, H. C., and H. Hidu. Effects of Pesticides on Embryonic Development of Clams and Oysters, and on Survival and Growth of the Larvae. US Fish and Wildl Serv, Fish Bull. 67:393-404. 1969.

27. Billard, R., and P. deKinkelin. Sterlisation Des Testicules De Guppies Par Des Doses Non Letales De Parathion. Ann Hydrobiol. 1:91-99. 1970.

28. Jensen, L. D., and A. R. Gaufin. Long-term Effects of Organic Insecticides on Two Species of Stonefly Naiads. Trans Am Fish Soc. 93:357-363. 1964.

29. Mount, D. I. and C. E. Stephan. A Method for Establishing Acceptable Toxicant Limits for Fish - Malathion and the Butoxyethanol Ester of 2,4-D. Trans Amer Fish Soc. 96:185-193. 1967.

30. American Public Health Association. Standard Methods for the Examination of Water and Wastewater. 13th ed. New York, APHA. 1971.

31. Mount, D. I. and W. A. Brungs. A Simplified Dosing Apparatus for Fish Toxicology Studies. Water Res. 1:21-29. 1967.

32. Drummond, R. A. and W. F. Dawson. An Inexpensive Method for Simulating Diel Patterns. Trans Am Fish Soc. 99(2):434-435. 1970.

33. McKim, J. M., and D. A. Benoit. Effect of Long-term Exposures to Copper on Survival, Reproduction, and Growth of Brook Trout Salvelinus fontinalis (Mitchell). J Fish Res Bd Canada. 28:655-662. 1971.

34. Weber, C. I., ed., Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. EPA - 670/4-73-001. July, 1973.

35. Benoit, D. A. Artificial Laboratory Spawning Substrate for Brook Trout (Salvelinus fontinalis Mitchell). Trans Am Fish Soc: 103(1): 144-145. 1974.

36. Litchfield, J. T., and F. Wilcoxon. A Simplified Method of Evaluating Dose-Effect Experiments. J Pharmacol Exper Therap. 96:99-113. 1949.

37. Thompson, W. R. Use of Moving Averages and Interpolation to Estimate Median Effective Dose. Bact Rev. 11:115-145. 1947.

38. Ellman, G. L., K. D. Courtney, V. Andres Jr., and R. M. Featherstone. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. Biochem Pharmacol. 7:88-95. 1961.

39. Hokanson, K. E. F., J. H. McCormick, B. R. Jones, and J. H. Tucker. Thermal Requirements for Maturation, Spawning, and Embryo Survival of the Brook Trout. J Fish Res Board Can. 30:975-984. 1973.

40. Pickering, Q. H., C. Henderson, and A. E. Lemke. The Toxicity of Organic Phosphorus Insecticides to Different Species of Warmwater Fishes. Trans Am Fish Soc. 91:175-184. 1962.

41. Henderson, C., Q. H. Pickering, and C. M. Tarzwell. The Toxicity of Organic Phosphorus and Chlorinated Hydrocarbon Insecticides to Fish. 1959 Sem Biol Probl Water Poll., R. A. Taft San Engr Ctr Tech Rpt W 60-3. 1960.

42. Tarzwell, C. M. Pollutional Effects of Organic Insecticides. Trans 24th Am Wildl Conf. p. 132-142. 1959.

43. Lewallen, L. L. and W. H. Wilder. Toxicity of Certain Organophosphorus and Carbamate Insecticides to Rainbow Trout. Mosquito News. 22:369-372. 1962.

44. Priester, L. E., Jr. Accumulation and Metabolism of DDT, Parathion, and Endrin by Aquatic Food-chain Organisms. Ph. D. Theisis. Clemson U. 1965.

45. Boyd, J. E. The Use of Daphnia magna in the Microbioassay of Insecticides. Ph. D. thesis, Penn State U. 1957.

46. Kadoum, A. M. Rapid New Method of Sample Cleanup for GC Analysis of Insecticide Residues. Bull Env Contam Toxicol. 2:264-273. 1967.

SECTION VIII

APPENDIXES

APPENDIX A - BIOASSAY DATA

Table A1. MEAN MONTHLY ANALYSES OF PARATHION
BLUEGILL CHRONIC TEST
(µg/l)

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Dec.	nil	nil	4.54	4.87	2.44	2.60	1.23	1.00	0.50	0.57	(9.19)	(9.63)
Jan.	0.01	0.01	4.40	4.10	2.12	2.37	1.06	1.04	0.45	0.40	(9.50)	(9.21)
Feb.	0.01	0.01	3.80	3.70	2.25	2.11	0.94	0.91	0.36	0.37	0.14	0.14
Mar.	0.02	0.02	4.77	4.74	2.44	2.27	1.30	1.31	0.58	0.61	0.17	0.18
Apr.	0.03	0.03	2.93	2.50	1.29	1.24	0.70	0.65	0.32	0.34	0.02	0.10
May	0.01	0.01	2.38	2.51	1.21	1.12	0.64	0.63	0.32	0.34	0.11	0.13
June	0.01	0.02	3.72	3.52	1.83	1.93	1.09	1.04	0.39	0.48	0.19	0.20
July	0.02	0.02	2.08	1.99	0.99	1.03	0.57	0.64	0.25	0.26	0.12	0.12
Aug.	0.01	0.01	3.03	3.07	1.55	1.54	0.75	0.75	0.44	0.46	0.19	0.23
Sept.	0.06	0.06	2.27	2.27	1.19	1.19	0.50	0.50	0.18	0.18	0.12	0.12
Oct.	0.07	0.07	4.96	4.96	1.74	1.74	0.57	0.57	0.22	0.22	0.13	0.13
Nov.	0.04	0.03	2.39	2.58	1.24	1.15	0.42	0.35	0.17	0.06	0.09	0.05
Dec.	0.02	0.02	2.44	2.44	1.87	1.87	0.80	0.80	0.27	0.27	0.06	0.06
Jan.	0.01	0.01	3.80	3.80	1.58	1.58	1.45	1.45	0.13	0.13	0.08	0.08
Feb.	0.04	0.04	1.81	1.81	1.26	1.26	0.47	0.47	0.15	0.15	0.08	0.08
Mar.	0.10	0.10	0.97	0.97	0.37	0.37	0.19	0.19	0.11	0.11	0.17	0.17
Apr.	0.17	0.17	3.29	3.29	1.86	1.86	1.18	1.18	0.31	0.31	0.40	0.40

Table A1. MEAN MONTHLY ANALYSES OF PARATHION (cont)
BLUEGILL CHRONIC TEST
(µg/l)

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
May	0.12	0.12	2.00	2.00	0.93	0.93	0.92	0.92	0.22	0.22	0.14	0.14
June	0.21	0.21	2.88	2.88	1.62	1.62	1.33	1.33	0.39	0.39	0.29	0.29
July	0.04	0.04	4.72	4.72	2.07	2.07	1.98	1.98	0.49	0.49	0.26	0.26
Aug.	0.19	0.20	3.77	2.78	2.16	1.97	1.33	1.33	0.56	0.56	0.38	0.38
Sept.	0.12	0.12	2.83	2.81	1.20	1.18	1.39	1.39	0.75	0.79	0.17	0.16
Oct.	0.03	0.0_	5.04	5.03	2.29	2.33	2.53	2.55	0.44	0.44	0.32	0.31
Mean[a]	0.06	0,06	3.23	3.14	1.53	1.59	1.00	1.00	0.34	0.34	0.16	0.17
SD[a]	0.09	0.09	1.88	1.83	0.98	1.03	0.74	0.74	0.26	0.26	0.14	0.14
High	0.39	0.39	10.59	10.59	4.23	3.95	3.01	3.01	1.03	1.08	1.10	1.10
Low	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

[a] based on weekly samples

Table A2. ROUTINE WATER QUALITY

Bluegill chronic test

	N	Mean	Range
D.O., mg/l	1008	6.1	3.8-7.3
pH	252	7.80	7.62-8.20
Alkalinity, mg/l	252	157	142-172
Acidity, mg/l	252	6.4	0.3-13.1
Hardness mg/l	252	265	158-309

Table A3. MEAN MONTHLY TEMPERATURES

Bluegill chronic test, degrees C.

Month	T	Month	T	Month	T
Jan.	23.0	Sept.	26.4	May	25.0
Feb.	24.2	Oct.	27.1	June	25.1
Mar.	25.0	Nov.	26.0	July	27.2
Apr.	24.7	Dec.	26.9	Aug.	27.2
May	25.6	Jan.	24.7	Sept.	26.6
June	26.1	Feb.	24.4	Oct.	
July	26.2	Mar.	24.4	Nov.	
Aug.	26.6	Apr.	25.7		

Table A4. BLUEGILL SURVIVAL-CHRONIC TEST

Tank Number

Date	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
71, Dec. 1	20	20	20	20	20	20	20	20	20	20	-	-
72, Mar. 1	20	20	17	13	20	17	17	17	20	20	49[a]	11[a]
May 1	19	20	17	12	20	17	17	17	19	20	34	10
Oct. 1	19	19	17	13	20	17	17	15	18	17	25	10
73, Mar. 1	18	19	17	13	20	17	17	15	14	14	12	10
May 1	13	17	17	13	20	17	16	15	9	13	12	10
July 1[b]	10	15	17	13	20	17	16	15	9	13	10	9
Nov. 1	9	9	8	8	11	9	9	9	8	9	7	7

[a]Eleven of same stock started in Tank 5B, Feb. 25, 1972.

49 from a new stock started in Tank 5A, Feb. 25, 1972.

[b]Later thinned to 3 males, 7 females per tank.

Table A5. BRAIN ACETYCHOLINESTERASE-
BLUEGILL 18 MONTH EXPOSURE
(μmol/min/g wet wt)

Tank	1	2	3	4	5	0
	1.26	3.14	3.03	6.34	7.35	12.97
	1.36	3.47	2.92	7.40	7.43	7.16
	1.28	4.07	3.22	5.88		5.07
	1.74	4.22	3.19	6.74		5.35
	1.23	4.98	2.10			6.04
	1.66	4.35	2.15			
	2.16	4.23	2.94			
	0.76	3.26	3.78			
	1.22	4.14	3.17			
	1.03	2.32	2.51			
			2.46			
Mean	1.37	3.35	2.86	6.59	7.39	7.32
% of Control	18.7	45.8	39.1	90.0	101	100

Table A6. BRAIN ACETYLCHOLINESTERASE -

BLUEGILL 23-MONTH EXPOSURE

(μmol/min/g wet wt)

Tank	1	2	3	4	5	0
	1.09	2.07	1.21	3.64	8.15	4.53
	1.15	1.41	1.75	5.37	5.85	9.76
	1.95	2.53	1.38	3.07	7.46	12.20
		1.92				7.49
Mean	1.40	1.98	1.45	4.03	7.15	8.50
% of Control	16.5	23.3	17.0	47.4	94.1	100

Table A7. BROOK TROUT ACUTE TEST

Number Surviving Per Tank

Hour	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
0	10	10	10	10	10	10	10	10	10	10	10	10
24	10	10	0	0	10	10	10	10	10	10	10	10
48	10	10	0	0	10	10	9	10	10	10	10	9
96	10	9	0	0	5	4	8	9	10	9	10	9

Analyzed Parathion Concentration, mg/l

0	nd	nd	3.30	3.06	1.59	1.59	0.88	0.91	0.43	0.47	0.21	0.25
48	nd	nd	4.84	4.60	1.96	2.11	1.06	1.00	0.51	0.52	0.24	0.28
96	nd	nd	4.41	3.79	1.94	1.96	1.04	0.86	0.43	0.42	0.25	0.26

Mean Weight of Fish, g

	81.1	83.8	86.0	78.2	83.6	89.6	73.1	82.4	68.4	87.7	74.3	82.9

Table A8. ROUTINE WATER QUALITY - BROOK TROUT CHRONIC

	N	Mean	Range
D.O., mg/l	504	8.2	6.7-10.5
pH	280	7.81	7.47-8.06
Alkalinity, mg/l	312	154	150-183
Acidity, mg/l	284	6.0	4.0-10.0
Hardness, mg/l	284	295	256-342

Table A9. MEAN MONTHLY TEMPERATURES - BROOK TROUT CHRONIC (±2°C)

Month	T	Month	T	Month	T
May	14.0	Sept.	16.7	Jan.	13.1
June	13.8	Oct.	15.0	Feb.	13.6
July	14.2	Nov.	13.4	March	14.2
Aug.	15.8	Dec.	13.3		

Table A10. MEAN MONTHLY ANALYSIS OF PARATHION IN WATER

BROOK TROUT CHRONIC TEST

(µg/l)

Month	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
May	0.07	0.07	9.50	8.40	3.41	3.27	1.84	1.83	0.86	0.94	0.40	0.46
June	0.04	0.05	6.12	6.40	2.70	2.57	1.59	1.53	0.63	0.63	0.46	0.44
July	0.04	0.03	9.49	9.52	4.43	4.50	2.94	2.84	0.76	1.07	0.59	0.62
Aug.	0.02	0.01	11.86	11.70	5.36	5.30	3.84	3.84	0.91	0.90	0.36	0.42
Sept.	0.02	0.02	1.32	1.32	0.80	0.80	0.59	0.59	0.31	0.31	0.11	0.11
Oct.	0.16	0.16	7.19	7.19	3.77	3.77	2.56	2.56	1.11	1.11	0.29	0.29
Nov.	0.02	0.02	3.68	4.84	2.16	2.54	0.94	0.89	0.83	0.83	0.21	0.21
Dec.	0.01	0.01	3.77	3.77	2.15	2.15	0.72	0.72	0.42	0.42	0.11	0.11
Jan.	0.01	0.01	6.62	6.62	3.06	3.06	2.13	2.13	1.12	1.12	0.13	0.13
Feb.	0.05	0.07	9.30	9.73	4.96	4.53	3.25	3.26	1.49	1.47	0.60	0.65
Mar.	0.01	0.01	12.34	12.34	6.03	6.02	4.43	4.43	1.92	1.92	1.07	1.07
Test mean	0.05	0.05	7.12	7.17	3.38	3.35	2.12	2.10	0.88	0.92	0.34	0.36
Number	45	45	45	45	45	45	45	45	45	45	45	45
SD	0.11	0.11	4.34	4.17	1.99	1.98	1.44	1.41	0.62	0.60	0.31	0.32
High	0.23	0.23	16.90	14.00	6.74	6.82	5.01	5.01	2.30	2.30	1.06	1.14
Lo..	nil	nil	0.28	0.28	0.62	0.62	0.06	0.06	0.19	0.19	0.02	0.01

Table A11. BROOK TROUT SURVIVAL IN CHRONIC EXPOSURE

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Date												
May 1	14	14	14	14	14	14	14	14	14	14	14	14
June 1	14	6	10	7	14	13	0[a]	12	13	13	14	14
July 1	14	5	8	6	14[b]	1[b]	0[b]	12	13	13	14	13
July 19	14	5	8	6	15	9	12	12	13	13	14	13
Aug. 1	14	4	8	6	15	7	9	12	7	12	14	13
Sept. 1	14	4	8	6	15	7	9	12	7	12	14	13
Oct. 1	14	4	8	6	12	7	8	12	6	12	14	13
Oct. 25[c]	14	4	8	6	12	6	7	12	6	12	14	13
Nov. 1	6	6	6	6	6	6	6	6	6	6	6	6
Dec. 1	6	6	6	6	6	6	6	4	5	6	6	6
Jan. 1	6	6	6	6	6	6	6	4	4	6	6	6
Feb. 1	6	6	5	6	5	6	6	4	4	5	6	6
Feb. 6	6	6	5	5	5	6	6	4	4	5	5	6

[a] Complete mortality from low flow and dissolved oxygen, May 27.

[b] One remaining fish from 2B moved to 2A on July 19.
Nine new fish added to 2B, twelve added to 3A.

[c] Thinning. All tanks reduced to 2 males, 4 females.
All fish from 2B and 3A removed. Six fish transferred from each corresponding duplicate.

Table A12. BROOK TROUT WEIGHTS - CHRONIC EXPOSURE

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
May 1												
Mean weight, g	63.9	62.8	60.6	62.6	60.6	67.6	60.0	68.2	67.0	59.3	59.9	63.0
Number	14	14	14	14	14	14	14	14	14	14	14	14
SD	12.8	11.1	8.8	13.6	6.9	9.5	8.4	8.7	10.5	8.1	8.2	10.0
Aug. 25												
Mean weight, g	182.3	201.9	179.7	204.2	163.8	-	-	198.2	209.0	184.9	172.4	183.3
Number	14	4	8	6	15	-	-	12	7	12	14	13
SD	32.8	64.8	42.5	25.2	17.4	-	-	19.2	28.6	21.5	20.4	31.9
Feb.												
Mean weight, g	331.7	439.7	347.6	436.2	370.2	290.5	394.5	320.8	358.8	337.8	351.2	416.2
Number	6	6	5	5	5	6	6	4	4	5	5	6
SD	80.7	78.3	72.8	78.6	119.9	47.7	100.6	17.3	149.7	63.7	45.9	74.6

Table A13. BROOK TROUT GONADOSOMATIC INDEX

Six-Month Exposure

(%)

Tank	OA	1A	2B	3A	4A	4B	5A	5B
Female	0.21	0.28	1.27	3.87	0.30		0.33	0.31
	0.34	0.24		3.11	0.36		9.75	0.34
				4.25			7.10	5.01
				5.65				2.74
Male	0.78		1.08	0.96	1.32	1.53	2.26	1.74
	0.53		1.90	2.38	1.19	1.29	7.16	0.39
	0.37		1.12	1.24			0.29	2.18
	2.88		1.88				1.73	
			1.25				2.51	

Table A14. BROOK TROUT GONADOSOMATIC INDEX

Nine-Month Exposure

(percent)

Tank	0A	0B	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Female	0.77	0.65	0.05	0.68	0.02	0.41	0.48	0.70	0.39	0.05	0.51	0.78
	0.51	0.48	0.58	0.62	0.04	nil	0.14	nil	0.49	nil	0.47	0.10
	1.26	nil	1.49	nil	0.67	1.00	0.46		0.53	0.70	0.33	0.50
	0.50	0.28	0.35	nil	0.43	0.63	0.58			0.57	0.50	0.42
					0.40	0.41						
Male	0.26	3.39	1.68	0.45		0.26	1.06	0.38	0.18	2.36	0.14	0.36
	0.34	0.56						0.70				0.08

Table A15. SUMMARY OF RESIDUE RESULTS FOR BLOOD AND MUSCLE OF TROUT EXPOSED TO PARATHION FOR 6 and 9 MONTHS.

Tank No.	Avg. Water Conc. ppb	No. of Fish	Months of Exposure	Residue, ppb		Conc. Ratio B/T
				Blood(B)	Tissue(T)	
21	7.1	2	6	1302	3842	0.34
		5	9	805	1260	0.64
22	3.4	3	6	788	1884	0.42
		5	9	361	435	0.83
23	2.1	5	6	603	1142	0.53
		5	9	256	306	0.84
24	0.90	3	6	126	419	0.30
		5	9	107	59	1.81
25	0.35	4	6	68	171	0.40
		5	9	43	52	0.83

Table A16. ROUTINE WATER QUALITY

Fathead Minnow Chronic Test

	N	Mean	Range
D.O., mg/l	384	7.1	8.1-6.3
pH	90	7.94	8.05-7.78
Alkalinity, mg/l	90	153	168-135
Acidity, mg/l	90	2.5	9.1-05
Hardness, mg/l	90	238	261-194

Table A17. MEAN MONTHLY WATER TEMPERATURE

Fathead Minnow Chronic Test

April	24.8	Sept.	24.2
May	23.9	Oct.	24.3
June	23.5	Nov.	23.5
July	23.8	Dec.	24.3
Aug.	23.2		

Table A 18. MEAN MONTHLY ANALYSIS OF PARATHION IN WATER

Fathead Minnow Chronic Test

(µg/l)

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
April	0.30	0.24	50.76	53.47	20.17	24.30	23.13	24.00	12.90	13.37	4.31	4.75
May	0.04	0.04	42.06	42.06	19.87	19.87	13.10	13.10	6.80	6.80	4.88	4.88
June	0.12	0.12	59.39	59.39	26.56	26.56	17.36	17.36	11.07	11.07	4.82	4.82
July	0.07	0.06	53.88	46.33	20.86	21.41	15.11	10.82	8.99	8.26	3.53	3.60
Aug.	0.11	0.06	41.17	42.94	18.08	19.96	13.28	14.57	8.77	7.78	4.98	5.56
Sept.	0.34	0.30	48.62	48.68	20.06	17.75	14.48	13.00	8.39	7.48	4.43	4.05
Oct.	0.06	0.06	40.25	39.06	18.82	18.31	10.85	10.58	5.37	5.39	3.39	3.41
Nov.	0.02	0.02	58.28	58.12	28.52	27.99	16.13	15.31	8.44	6.83	2.64	3.85
Dec.	0.02	0.04	55.56	53.25	27.07	23.50	15.00	12.21	7.98	8.18	4.25	3.97
Mean	0.14	0.12	49.40	48.53	21.51	21.94	15.61	15.36	8.95	9.02	4.46	4.36
SD	0.14	0.18	18.08	15.06	8.16	7.18	7.55	7.40	4.14	2.87	1.55	1.18
High	0.82	0.82	104.20	104.20	44.86	44.86	38.70	38.70	17.90	17.90	7.30	7.30
Low	nil	nil	8.61	16.81	3.19	6.70	2.06	3.34	2.81	2.24	1.13	1.60

Table A 19. GROWTH AND SURVIVAL OF FATHEAD MINNOWS IN PARATHION

Tank	30 days		60 days		
	Number present	Mean length, mm	Number present	Mean length, mm	Mean growth, mm
OA	20	14.7	19	22.6	7.9
OB	33	14.6	28	22.3	7.7
1A	27	13.8	23	20.2	6.4
1B	28	15.2	27	23.0	7.8
2A	24	14.9	24	19.7	4.8
2B	28	14.1	27	19.6	5.5
3A	25	15.5	23	23.2	7.7
3B	28	13.8	26	19.3	5.5
4A	26	16.0	23	22.4	6.4
4B	31	14.5	29	20.4	5.9
5A	23	16.4	23	23.3	6.9
5B	26	14.8	22	23.8	9.0

number at start = 35 per tank

Table A20. D. MAGNA ACUTE TEST

Flow-through, 18°C

Number Surviving

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B
Start	10	10	10	10	10	10	10	10	10	10
24 hr	10	10	7	7	10	7	7	10	9	9
48 hr	10	10	0	0	8	4	7	6	9	9
72 hr	10	10	0	0	8	3	7	5	9	9
96 hr	10	10	0	0	5	3	7	4	9	9
Parathion, µg/l	0.11	0.10	1.56	1.55	0.85	0.86	0.45	0.44	0.23	0.24

LC50, µg/l 24 hr = 2.70 (5.61 - 1.30)
48 hr = 1.00 (1.73 - 0.58)
96 hr = 0.62 (0.90 - 0.43)

Table A 21. D. MAGNA CHRONIC TEST

Number Surviving

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Start	10	10	10	10	10	10	10	10	10	10	10	10
1 wk	10 A 89 Y	9A 34Y	0	0	0	0	10	9	10	10	10	10
2 wk	8 A 476 Y	6A 589Y	0	0	0	0	8A 271Y	8A 233Y	10 A 387 Y	9A 219Y	10A 375Y	10 A 416 Y
3 wk	7 A 205Y	3A 51Y	0	0	0	0	OA 103 Y	OA 176Y	9 A 268 Y	8A 280Y	10A 114Y	9 A 367 Y
Total young	770	674	-	-	-	-	374	409	655	499	489	783

Parathion Analysis, µg/l

Start	0.01	0.02	0.80	0.79	0.37	0.38	0.28	0.27	0.16	0.16	0.09	0.09
1 wk	0.02	0.02	0.72	0.70	0.31	0.30	0.20	0.20	0.10	0.10	0.08	0.08
2 wk	0.02	0.02	0.85	0.85	0.48	0.47	0.23	0.23	0.10	0.10	0.08	0.07

LC50, µg/l

1 wk = 0.28 (0.29 - 0.26)
2 wk = 0.25 (0.22 - 0.28)
3 wk = 0.14 (0.16 - 0.13)

RI50 = 0.24 (0.32 - 0.17)
MATC = 0.08 µg/l

Adults - A
Young - Y

Table A22. GAMMARUS ACUTE TEST

Number Surviving

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B
Start	10	10	10	10	10	10	10	10	10	10
24 hr	10	10	6	5	7	6	10	10	10	10
48 hr	10	10	0	0	0	0	9	10	10	10
72 hr	10	10	0	0	0	0	8	7	9	10
96 hr	10	10	0	0	0	0	5	5	8	10
Parathion, µg/l	0.08	0.09	2.12	2.09	1.13	1.14	0.44	0.43	0.19	0.19

LC50, µg/l - 24 hr = 2.1 (3.8 - 1.2) for juveniles at 20°C
48 hr = 0.62
96 hr = 0.43 (0.65 - 0.29)

Table A23. GAMMARUS SUB-CHRONIC TEST

20°C

Number Surviving

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B
Start	20	20	20	20	20	20	20	20	20	20
8 days	20	20	0	14	16	17	16	15	19	18
23 days	16	19	0	0	1	0	4	7	15	13
30 days	15	15	0	0	0	0	0	1	6	7
36 days	13	14A[a] 8Y	0	0	0	0	0	1	6A[a] 5Y	7A 4Y
43 days	13	14A	0	0	0	0	0	1	3A	7A
Mean parathion conc. µg/l	0.03	0.02	1.64	1.65	0.24	0.24	0.15	0.15	0.04	0.05

LC50 - 43 days = 0.07 (0.50 - 0.01) µg/l

[a] Adults - A
young - Y

Table A24. C. TENTANS ACUTE TEST

Number Surviving

Tank	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
Start	10	10	10	10	10	10	10	10	10	10	10	10
24 hr	10	10	10	10	10	10	10	10	10	10	10	10
48 hr	10	10	5	8	6	9	10	10	10	10	10	10
72 hr	10	9	2	2	5	7	9	9	10	8	10	10
96 hr	10	9	0	0	5	4	9	8	9	8	10	10

Analyzed Parathion, µg/l

Start	0.14	0.14	66.3	66.3	33.6	33.6	17.6	17.6	5.7	5.7	5.8	5.8
72 hr	0.09	0.09	48.6	48.6	33.0	33.0	17.2	17.2	6.2	6.2	4.0	4.0

LC50, µg/l 48 hr = 135 (703 - 26) for 4th instar larvae at 21°C
96 hr = 31.0 (43.4 - 22.1)

Table A25. C. TENTANS SUB-CHRONIC TEST

Number Surviving

Tank	OA	OB	1A	1B	2A	2B	3A	3C	4A	4B	5A	5B
Start	10	10	10	10	10	10	10	10	10	10	10	10
4 days	10	10	6	8	10	8	10	9	9	10	9	9
7 days	10		0	4	8	6	7	9	8	6	8	7
14 days	10E	10E	0	0	0	0	3	4	3	1	3	4
Mean parathion conc., ug/l	0.3	0.3	52.9	52.9	23.6	23.6	11.0	11.0	5.0	5.1	3.1	3.1

E = emerged

LC50 - 14 days = 2.3 (3.4 - 1.6) ug/l

APPENDIX B - BROWN TROUT TESTS

Young brown trout, Salmo trutta Linnaeus, 16 to 19 cm total length, were acutely exposed to parathion in an LC50 test and also in a residue uptake test. The acute LC50 test method was identical to that of the brook trout. The LC50 results for 24 and 96 hours (Table B1) show essential agreement with those for brook trout. Symptoms of parathion poisoning were also similar.

In a separate test, brown trout of the same stock were exposed to the two highest parathion levels which had caused no acute mortalities - 0.68 and 0.47 mg/l. A total of 45 fish were used in each of the two tanks. During the parathion exposure period, three fish were removed from each tank at selected intervals up to 64 hours. At 65 hours, the remaining fish were transferred to two duplicate tanks with uncontaminated flowing water. Again three fish per tank were removed at time intervals until all fish were gone at 79 hours. Muscle samples dissected from the dorsal fin region were preserved at -18°C and later analyzed for parathion residues. The residue values shown in Table B2 are each the mean of true duplicates. Precision for the clean-up and chromatographic method was ±5%.

A simple model was applied to the data from the 0.47 mg/l exposure and plotted (Figure B1). Assuming first order kinetics, the change in fish residue, C, can be expressed as a function of C_w, the toxicant concentration in water:

$$dc/dt = k_1 C_w - k_2 C$$

$$C = k_1 C_w / k_2 + ((C_o - k_1 C_w) / k_2) e^{-k_2 t}$$

where k_1 is the rate constant for uptake from water, k_2 is the rate of elimination, metabolism, or other removal, and C_o is the initial fish residue. The washout data were fitted to a least-squares line of log C vs. time, giving $k_2 = 0.002\ hr^{-1}$. Substituting for k_2 and C at 64 hours, the value of k_1 is about $1.0\ hr^{-1}$. The calculated steady state level of parathion in the muscle is 250 mg/kg, reached in about 90 days, assuming no mortality or change in mechanism. The concentration factor of 500 then agrees very well with the concentration factors for chronically exposed brook trout muscle. Although the data are not as precise as one would like, they show that a simple model can be used to describe parathion uptake in fish. The rate constants would, of course, vary with the temperature, fish species, and perhaps, order of magnitude of the dosage.

Table B1. BROWN TROUT ACUTE TEST

Number Surviving Per Tank

Hour	OA	OB	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
0	10	10	10	10	10	10	10	10	10	10	10	10
24	9	10	6	0	10	10	10	10	10	10	10	10
48	9	10	0	0	9	10	10	10	10	10	10	10
96	9	10	0	0	5	1	10	10	10	10	10	10
144	9	10	0	0	0	0	9	6	10	1	10	10

Analyzed Parathion Concentration, mg/l

Pooled daily	nd	nd	2.40	2.40	1.63	1.63	1.04	1.04	0.68	0.68	0.47	0.47

Brown Trout Acute LC50

Hour	LC50, mg/l	Upper CL, mg/l	Lower CL, mg/l
24	2.15	2.34	1.97
48	1.93	2.01	1.85
96	1.51	1.65	1.38
144	1.13	1.03	1.24

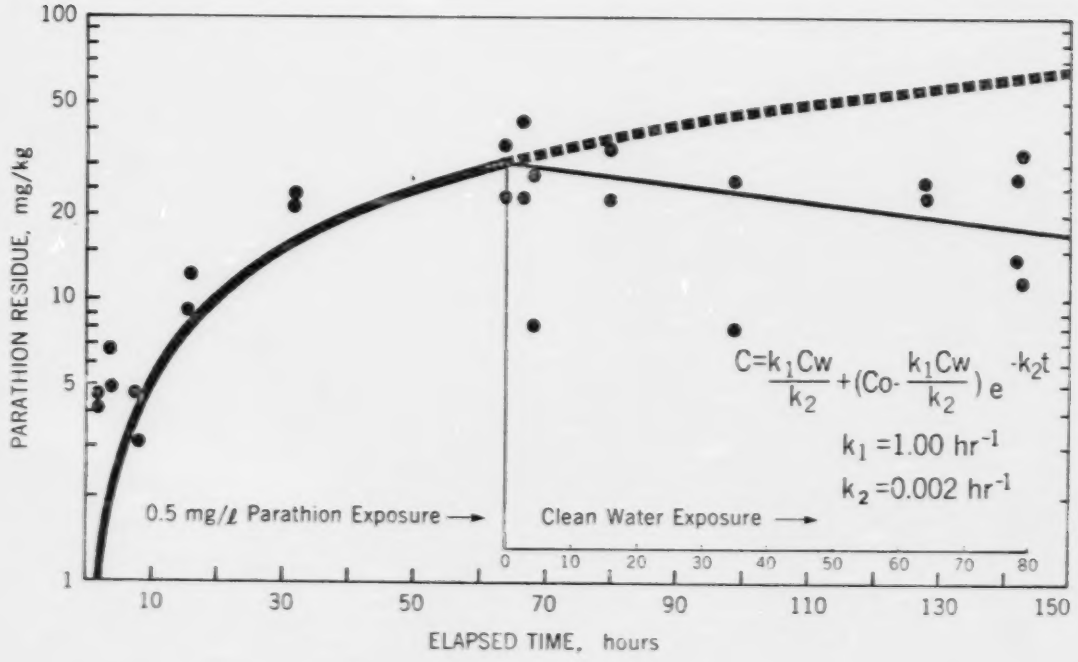
Table B2. PARATHION RESIDUES IN BROWN TROUT MUSCLE

Parathion in water, mg/l		0.47	0.68
Uptake residues, mg/kg	2 hr	4.10	5.48
		4.53	10.61
	4 hr	4.87	7.48
		6.48	
	8 hr	3.11	10.91
		4.63	12.45
	16 hr	9.08	7.98
		11.95	16.66
	32 hr	21.78	15.79
		23.64	43.34
	64 hr	22.88	46.49
		34.80	57.99
Washout residues, mg/kg	2 hr		64.05
			72.85
	3 hr	22.74	
		42.21	
	4 hr		29.44
			66.92
			33.24
	4.5 hr	8.27	
		27.66	
	16 hr	22.38	41.78
		33.94	44.79
	35 hr	7.90	22.53
		26.23	42.50
	64 hr	23.14	21.29
		25.60	55.74
	77 hr		28.13
			22.76
			44.52
	78 hr	13.89	18.96
		27.38	48.35
	79 hr	12.59	36.20
		32.77	47.96

True duplicates analyzed at each time period.

Precision ± 5%

Parathion uptake and washout in brown trout muscle, 12°C



APPENDIX C - CHLORINATED HYDROCARBON PESTICIDES IN FISH FOOD

Samples of fish food used for chronic tests were analyzed for chlorinated hydrocarbon pesticides. Initial attempts to attain effective clean-up using standard Florisil chromatography failed with these food samples. We subsequently used the silicic acid chromatography procedure reported by Kadoum.[46] Food samples were extracted with hexane in the presence of Na_2SO_4. Concentrated extracts were applied to columns of silicic acid (Mallinkrodt 100 mesh, chromatography grade) and eluted with hexane and 2%, 10%, 40%, and 70% benzene in hexane and benzene. Fractions were pooled, evaporated and rechromatographed a second time with collection of individual fractions: hexane, 2% benzene, 10% benzene, 40% benzene, 70% benzene, and benzene. These were analyzed by GC on 3% OV - 17 on Chromosorb W at 195°C using electron capture detection. Standard solutions of 13 chlorinated hydrocarbon pesticides were chromatographed to provide retention times (RT) for identification purposes and peak areas (as counts) for quantification. The conditions of elution from the silicic acid and the GC RT's were used for tentative identifications. Good matches, however, were not obtained for heptachlor epoxide and lindane and nearest peaks were used; all peaks eluted in the DDT and DDT degradation product area were summed and calculated as DDT + DDD + DDE. These, therefore, are maximum values. From the blood samples, resolvable peaks distinct from the interferences could not be obtained in the retention time regions of dieldrin or endrin in the 70% benzene fraction. The presence at low level (ppb) or absence of these pesticides could not be verified. The overall results are summarized in Table C1. In general we conclude that chlorinated hydrocarbon pesticide levels were low in each food source used and should not have significantly affected our tests.

Table C1. PESTICIDE RESIDUES IN FISH FOOD

(μg/kg)

Food Sample	Aldrin	Heptachlor	DDE DDD DDT	Heptachlor Epoxide[b]	Lindane[c]	Dieldrin Endrin[d]
Starter Mash	<1	<0.1	<0.5	nil	<0.3	ND
No. 3 Pellet	nil	<0.2	<0.5	nil	<0.3	ND
No. 6 Pellet	nil	<0.1	<0.5	nil	<0.6	ND
No. 5 Pellet	(a)	<0.1	<0.5	<1	<1	ND

(a) a GLC peak near the Retention time (RT) of aldrin, but probably not aldrin, gave a value of 10-15 ppb calc. on the basis of aldrin.

(b) No true match of R.T. with standard; nearest GLC peak taken as Heptachlor epoxide for calc. of No. 5 sample.

(c) No true match of R.T. with standard; nearest peak taken as lindane for calc. R.T. was 256 sec. vs. 249 sec. for lindane stdz. Peak may be a phthalate ester.

(d) These pesticides could not be detected (ND) unless present at very high levels because of broad peaks of relatively high level interferences present in the 70% benzene fraction.